STUDIES IN ANIMAL PIGMENTATION.

By

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STUDIES IN ANIMAL PIGMENTATION.

I. GENERAL INTRODUCTION.

Every system in the body is more or less intimately associated with some other system or systems so that a definite interdependence is established. Many of the factors controlling the functioning of these systems, and the extent to which one system may influence another, are still obscure and this is particularly true in the case of the pigmentary system. Although on occasion it may respond readily to external stimuli, and is always greatly influenced by environmental conditions, it is fundamentally as definite an entity as any other. Because of its close association with other vital body functions and its extreme activity during certain pathological conditions, it is highly desirable that the mechanism of its functioning should be investigated and its importance in the body estimated.

The problem of pigmentation has been the subject of/

of many investigations, which, however, have dealt mainly with the manifestation of colour in its final form, and it is only comparatively recently that an attempt has been made to study the fundamental processes involved in pigment formation and to determine the nature and origin of its controlling factors. In this thesis the problem has been approached from chemical, physical and physiological standpoints, for it is recognised that whilst the genetic basis of pigmentation is of primary importance, those factors controlling the extent to which the gene may find expression are in all probability environmental and require further analysis.

In the present study of animal pigmentation an attempt has been made to define some of the physiological factors which control the expression of the pigmentary system and to elucidate the underlying mechanism. With this end in view, a histological survey of the skin and wool of the Suffolk breed of sheep was undertaken and a study of the phenomenon of colour banding in the fleece of various other breeds was made. A natural extension of this work was the experimental study of the individuality of pigment producing cells, after differentiation. For the sake of convenience mice replaced sheep in these experiments. Following Roberts' (78-83) work on the inheritance/

inheritance of colour and colour patterns in the sheep, an attempt was made by means of the Dopa reaction technique to demonstrate the nature of the inhibitors and anti-inhibitors which are believed to be the modifying factors in the production of the various colours. As a preliminary to this, a series of in vitro experiments was carried out and the behaviour of the reaction under varying conditions investigated.

In the following studies it has been necessary to refer occasionally to pigmentation in the Invertebrates while among the Vertebrates certain comparisons have been made with Amphibia, Pisces, Reptilia and Aves, but the main thesis is concerned with pigmentation in Mammalia, and more specially in the sheep.

Section II - The first part - is confined to a description and discussion of experiments of a fundamental nature, whilst in Section III the problem of pigmentation in so far as the sheep is concerned is discussed.

II. (a) THE PIGMENTARY SYSTEM.

Pigment occurs in every group in the animal kingdom and its origin and precise nature have for years been the subject of much research. In mammals the characteristic pigment, melanin, occurs normally in the skin and its derivatives, the eye and certain parts of the nervous system. It also occurs in some pathological conditions of the ovary, uterus, adrenals, rib cartilages, peritoneum and in rapidly growing melanotic tumours.

It is usual to consider the physiological mechanisms in the body which produce melanin under the collective title of "the pigmentary system" without reference to the actual site in the body where that mechanism is functioning. The constituent elements of this mechanism are always the same no matter where melanin is being produced, but they may vary quantitatively and qualitatively within limits. (It should be clearly recognised that the use of the phrase "pigmentary system" does not include those varieties of pigment derived from blood, bile or urine.) The pigmentary system itself essentially consists of a basic chromogen and a catalytic enzyme or enzymes, and it is the interaction of these which produces melanin. The nature of the basic chromogen and/

and the catalytic enzymes still gives rise to controversy, but recent work (Bloch and Eppinger) has made it practically certain that tyrosin is the most commonly occurring chromogenic substance and that upon its oxidation by enzyme action melanin is produced. It has been shown that the action of tyrosinase on tyrosin produces a red pigment which in alkaline solutions oxidises spontaneously to a colourless substance, but which in the presence of molecular oxygen or a specific oxidising agent in the cell is converted into melanin. Raper and Wormall (76) showed that on oxidation dihydroxyphenylalanine also produced melanin, and from this it has been concluded that dihydroxyphenylalanine may be accepted as an intermediate product in melanin formation. (See Table I). Unlike tyrosin, dihydroxyphenylalanine can be converted into melanin in the epidermis and for this reason, "dopa", as it is called, has been employed to test the pigment forming ability of epidermal cells. In mammals, these pigment forming cells are situated in the lowermost part of the epidermis, the Malpighian layer, and with suitable staining will give a precise and specific reaction. As yet it has not been possible to isolate those specific oxidases concerned in melanin production, and practically nothing is known/

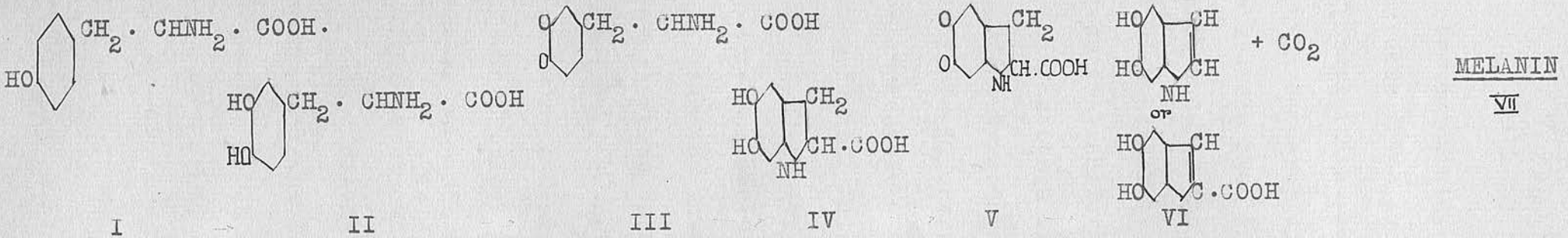
[contd. page 8]

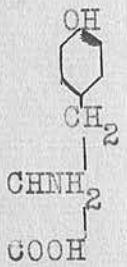
Table 1

(a) Stages I and II take place in the blood, and then Dopa as such in circulation may be supplied to the melanin producing tissues. Stages III, IV and V probably take place within the cells, and when Stage V. is reached and the red substance is produced no further enzyme action is necessary. The final product Melanin which immediately follows Stage VI, is the result of oxidation of the red substance. From this it can readily be seen that in melanogenesis the intracellular environment is of great importance.

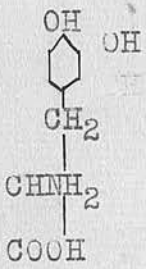
The interval between the formation of dopa and the production of the red substance offers wide opportunity for interference in the functioning of the pigmentary system; and some break-down or deficiency in the chemico-physical reaction at one or other of these stages offers a better explanation for localisation or restriction of pigment production than does the postulation of inhibitors or anti-inhibitors as specific entities.

(a)

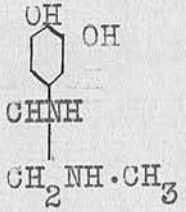




Tyrosin



3:4-Dihydroxyphenylalanine



Adrenalin

(b) To indicate the possibility of Tyrosin being the common source of melanin and adrenalin.

TABLE I

known concerning them. It seems however that the oxidase which transforms tyrosin to dihydroxyhenylalanine is distinct and different from that which converts dopa into melanin and must occur elsewhere than in the epidermal cells, for tyrosin must undergo the preliminary change before it reaches the epidermal tissues, the cells of which are powerless to transform tyrosin directly into melanin.

The steps in the production of melanin from its source, tyrosin, can be best illustrated by reference to Table I, where the successive stages in melanogenesis are represented by chemical formulae from Pryde (72).

Pigment is deposited either in the form of discrete granules or in a diffuse state, and commonly although not invariably the two types occur together. The view has been expressed many times that three distinct pigments exist: red, yellow and brown (Wentworth, Winge, Walther), or yellow, brown and black (Little), but in certain in vitro experiments (Pages 30 - 41) with dopa it was possible to produce every shade of colour from red and yellow to brown and black, and this would seem therefore to dispose of the theoretical existence of more than one pigment. This one pigment however may vary considerably in its colour expression, depending on chemical/

chemical and physical factors. When distinct colours such as red, brown or black which characterise the coat of certain breeds of cattle are considered, the difference between each is striking and unmistakable, but if these same colours be examined microscopically the fact emerges that each is fundamentally identical with the other. The individual pigment granules are similar, and it is their disposition in the fibre cells, their quantity, association with diffuse pigment, and the accomplished degree of oxidation which materially affect the colour that is obvious to the naked eye. It is certain also that the physical structure of the fibre vastly influences the final visible colour.

Criticism has been raised concerning the reality of the existence of only one pigment, since breed colours are apparently so rigid and distinct. The explanation for the maintenance of these distinct colour differences is a simple one; since every colour expression is genetic, the differences in genetic constitution between breeds determine also the extent to which the colour genes shall find expression. By appropriate stimuli an animal which is normally red could be made to exhibit black coloration and so on. This experimental interference naturally can have only an extremely limited application/

application.

Gortner (22) found that the black pigment in the wool of sheep differed from that of the black rabbit in the quantity of residue after treatment with NaOH but this seems to have been a quantitative and not a qualitative difference. According to Wiedmer's (19) researches light colour in horses is due to the absence of granular pigment and the presence of diffuse pigment. In blackhaired horses granular pigment is always greatly in excess of the diffuse type. The same is true in man. Granular pigment is very stable and is known to be acid insoluble differing greatly in this respect from diffuse pigment which is easily soluble in acid.

The disposition and distribution of the pigment granules in fibres, are determined primarily by the cellular structure of the particular fibre and by the rate at which the pigment is produced. In lightly pigmented fibres the pigment may be restricted to the medulla, but equally often it is found scattered through the cortex, and in heavily pigmented fibres both cortex and medulla contain pigment.

Hausman (32) postulates a different form and mode of orientation of granules for the hair of different species, and apparently does not accept the fact that variations in fibre structure are

sufficiently/

sufficiently responsible for such differences.

The results of this study are in accordance with the observations of Haugg that colour tones in hair are quantitative and not qualitative and are due to the arrangement and distribution of the pigment in the hair follicle. The precise colour of pigment is dependent on various factors; observations show that depth of colour is due to variations in quantity and disposition of the pigment which are determined by prevalent chemical factors present in the body during melanogenesis.

Both the fundamental chromogen and the oxidases may be present and yet melanin formation may not result, for in certain cases their interaction is rendered ineffective or impossible through the presence of inhibitors. The experiments with dopa suggest that an inhibitor is not necessarily a specific substance and that the effects attributed to it are due to quantitative and qualitative differences in the constituents of the pigmentary system. Przibram (73) is inclined to this view.

Melanin in tissue may exhibit every shade of colour from palest buff to most intense black. Genetic black is the most stable form of pigmentation, red the least and the latter shows the greatest susceptibility to dilution factors. Every gradation between/

between colourlessness and intense pigmentation may be found and in wool fibres whiteness may be due to:

1. Absence of melanin;
2. Insufficiency of melanin;
3. Presence of "white" melanin;
4. Physical properties concerned with the structure of the fibre by means of which light rays are reflected.

Spiegler's (19) theory that white melanin was present in white horse hair has not received credence since Gortner's experiments failed to confirm his deductions. Nathusius (19) however demonstrated white granular pigment in the medullary cells of certain white hairs from sheep which he believed were pigment or nearly related granules. These have since been observed in the white hairs of certain rabbits and are specially obvious after staining with Nissl's Methyl blue. This white melanin may be the result of over-oxidation. A comparable phenomenon is seen in the bleaching or weathering which occurs in the tips of certain dark wools and in the coats of certain cattle. In these cases the lighter colour is due partly to pigment disintegration initiated by exposure to excessive light and partly to continued oxidation through the agency of the free oxygen of the air.

The natural bright brown or fawn coloration which is sometimes seen in the exposed portions of the fleeces of black sheep can be reproduced exactly by the/

the application of hydrogen peroxide to the intensely pigmented portions of black fibres.

It is necessary to distinguish between this fading effect of the tips of exposed fibres and the occurrence of what are frequently called "agouti tipped" fibres growing on the hair-bearing parts of black sheep - e.g. face, legs, etc., in these agouti fibres the golden-red pigmentation of the tip is constant from the outset. It is succeeded by intense black pigmentation lower down on the shaft of each fibre, which resists weathering and oxidation and never loses its jet black appearance.

The seasonal whitening which takes place in such animals as Lepus Americanus has been ascribed to an acute and complete bleaching of the pigment granules themselves, (Hadwen) and not to the shedding of the coat as a whole. Atmospheric oxygen is powerless to produce such an exaggerated change and since there is no known connection between the living body cells and the wholly dead keratinized cells of the fibres it is difficult to reconcile these facts with the theory. It is more probable that the whitening is due to the gradual replacement of the old coloured coat by a new white one, without mass shedding of the coloured coat as a whole. Metchnikoff's (10) belief in the reality of pigmentophagic cells which in greying individuals wandered/

wandered up the medulla of the fibre and ingested the cortical pigment granules, thus leaving the fibre colourless, is now only historically interesting. His illustrations of pigmentophagic cells bear a startling likeness to certain types of ordinary commonly occurring pigment cells known as dendritic cells (See Fig. 13). It is only whilst melanin is in the living cell that it may be influenced by physiological conditions of the body, and melanin deposited in fibres is no longer controlled by body conditions, so that it is not possible to accept the suggestion that over-night greying or sudden whitening of hair, due to nerve shock, does occur.

The precise origin of pigment granules is still a much discussed question. Their intracellular origin is undoubted, but beyond this no satisfactory hypothesis has yet been advanced. Recent tissue culture work (Koller) suggests that pigment granules arise de novo in the cytoplasm but other workers believe that mitochondria are transformed into granules and a third school of workers supports the theory of the nuclear origin of pigment. No one has yet succeeded in satisfactorily demonstrating the rôle of the nucleus in melanin formation, and whilst the results of Ludford's (58) research on the melanotic sarcoma of/

of the horse definitely supports this theory, Miescher's (62) work on the eye of the chick, rabbit and guinea pig as definitely refutes it. Jeliasowa Paspalewa (43) affirms that in the eyes of sheep, fowls, frogs and toads, pigment is nuclear in its origin, and even states that during mitosis whole chromosomes may become melanised. Makarov (59) puts forward an interesting theory concerning the role of the chondriosomes in pigment formation. He states that the chondriosomes function as pro-pigment nuclei, becoming steeped in the basophilic mother substance of pigment. They are supposed to act on substances entering the cell and he states that neither the golgi apparatus nor the cell nucleus nor the nuclear bodies take any part in pigment formation. Observations on the epidermal pigment of sheep suggest that there is some close connection between melanin formation and the nucleus. Very often the nucleus of a cell is practically obscured by pigment granules which cover it in such a way as to form a double cap. (See page 102). These nuclear caps, single or double, have been recorded in man (Percival and Stewart, 70) cattle (Esskuchen, 19), pigs (Teodoreanu, 93), horses (Ludford, 58) and we have recently observed them in many breeds of sheep. This disposition round the nucleus is certainly not fortuitous and that this pigment has a protective/

protective function is certain. It is also possible that the aggregations indicate the site of origin. That is to say, the melanin is nuclear in origin in that the oxidases which convert the dihydroxyphenylalanine of the cytoplasm into melanin are elaborated by the nucleus itself. It is important to note that pigment production is always preceded by cellular activity, which ultimately depends on nuclear activity. Sewall Wright (99) in 1917 suggested that the oxidising agents which produced pigment were secreted by the nucleus, and to-day it seems that this is possibly true. Equally probable is the suggestion that nuclear activity may control the intracellular environment by a quantitative and qualitative discharge into the cytoplasm of such substances as would act as inhibitors, or intensifiers, of pigment production. Sewall Wright thought that it was the oxidase which determined the precise colour, but in vitro experimentation has shown that this is not the case and that colour is determined by those factors governing the interaction of the chromogen and the oxidases. The question then arises, why is it that the nucleus of one cell may behave quite differently from that of its neighbour? Only a difference in genetic constitution can account for these variations. From the analytical studies carried out on the skin of variously-coloured sheep/

sheep it seems that some cells are more potentially pigment-producing than others, and just as there is in animals every grade between albinism and full self-colour so is there every grade between the cell which cannot produce pigment and the cell which produces it to a maximum degree.

A description of the experimental standardisation of the dopa reaction technique which was used in these studies accompanies the account of the pigmentogenic potentiality of the epidermis of the sheep. (See pages 30 - 41).

II. (b) THE DOPA REACTION.

The "Dopa" reaction was first described by Bloch. He showed that when sections of skin were left in contact with 3-4-dihydroxyphenylalanine (dopa), melanin was produced in the epidermal cells. This melanin was distinct from and uninfluenced by already existing melanin present in the skin or its associated fibres. The use of the reaction may be looked upon as a method of revealing the maximum amount of pigment which the skin is capable of producing, or of realising the full pigment potentiality of the epidermal pigment cells. The reaction always took place in those parts of the skin which were normally pigmented (i.e. Malpighian layer) and was greater in heavily pigmented skin than in lightly pigmented skin. He concluded from this that any cell which produced melanin necessarily contained an oxidase which was capable of oxidising dopa and which was specific in its action. This he called dopa oxidase. Percival and Stewart (1930) have reviewed all the recent investigations on melanogenesis and have given a concise account of the present position of the Dopa oxidase theory. Our acceptance of dopa as a suitable substrate in melanin formation has been the basis of the following research. Roberts' work on the genetics of colour inheritance in the/

the sheep, suggested to us that the use of a chemical test in genetic analysis would probably yield interesting results and with this end in view an investigation of the dopa reaction in the skin of the sheep was undertaken.

Several research workers have used various skin extracts (Durham, Onslow, Przibram) to demonstrate the presence or absence of oxidising enzymes, and the existence of anti-enzymes or inhibitors. Young animals were always used since it was apparently impossible to extract any active enzymes from the skin of older animals. A search of the literature has not revealed any work of this nature in sheep and it was thought that in the initial stages at least a study of the dopa reaction in skin sections would provide more information than would the skin extract method.

IIb. (i) Findings with Dopa in sections of lamb and adult sheep skin.

The material selected was classified strictly according to the age and colour of the sheep.

The Dopa reaction technique is itself very simple but the water used in preparing the dopa solution must be carefully distilled and the pH accurately adjusted (Percival and Stewart describe this method of preparation in detail). Fresh skin samples are fixed in 5% neutral formalin for four or five hours and then frozen/

frozen sections are cut. These sections are then placed in dopa solution (1 mg. dopa to 1 cc. prepared water; pH 7.34) and incubated for three hours at 37°C or for twenty-four hours at room temperature. Experience showed that eighteen hours at room temperature gave the optimum results and in our work with sections of the skin of the sheep this procedure was always followed.

The following two series are illustrative of the type of material used in the preparation of these sections.

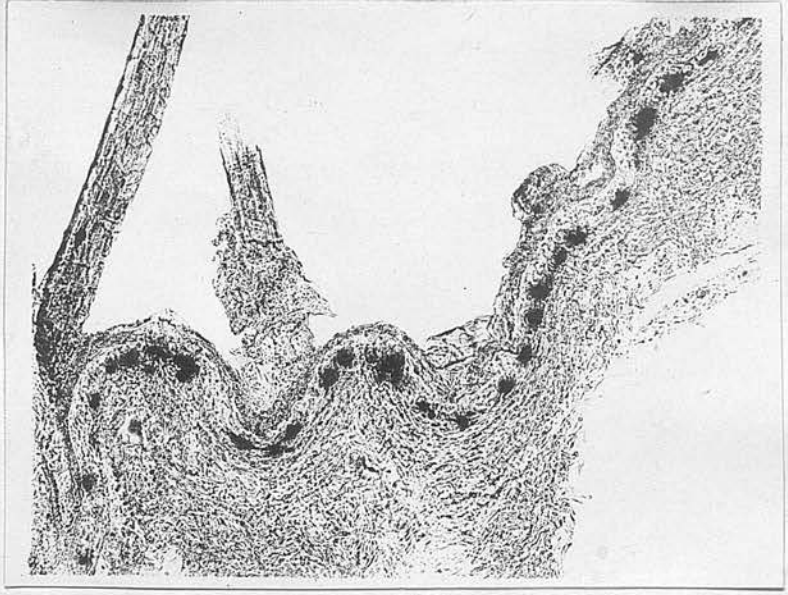
Series I. Samples from lambs not more than two or three days old.

1. Recessive brown.
2. Recessive black.
3. Badgerface - white portion.
4. Badgerface - black portion.
5. Reversed badgerface - black portion.
6. " " - white portion.
7. White spot from black lamb - dominant white.
8. Dominant black.

Series II. Samples from adult sheep.

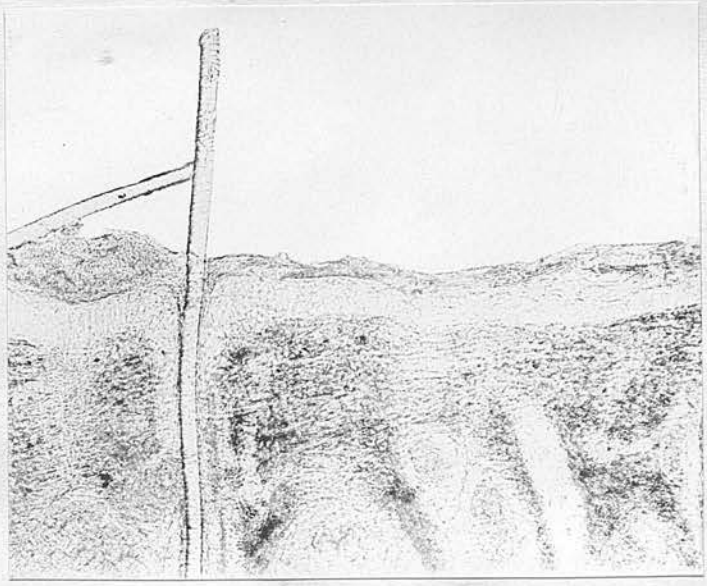
1. Recessive brown.
2. Heterozygous white (white x brown, F1)
3. Recessive black.
4. Black with isolated white spot (Pattern)
5. Reversed badgerface (wool showing banding)
6. Dominant white.
7. Brown and white area from face of Gritstone x Cheviot.
8. Dominant white (aged).

Dopa staining does not in any way affect the formed pigment but it reveals those cells which are potentially/



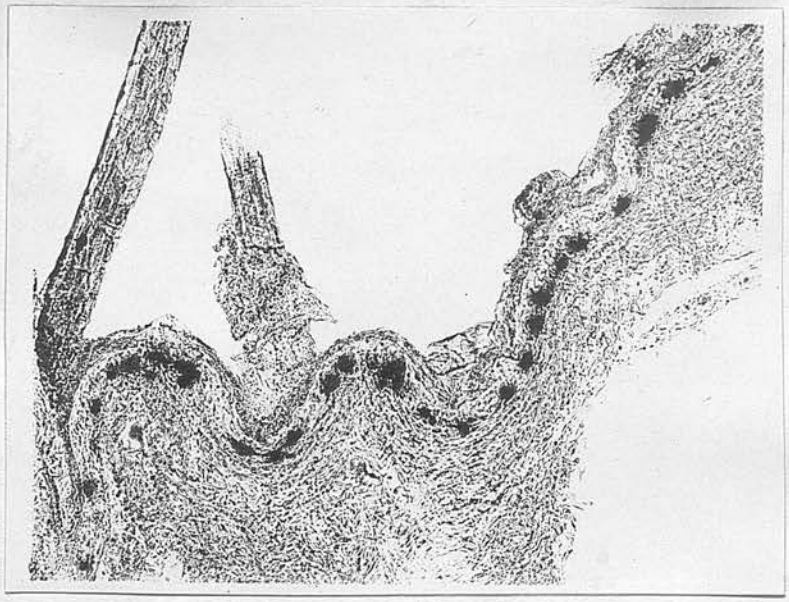
Recessive Brown

Badger face White portion



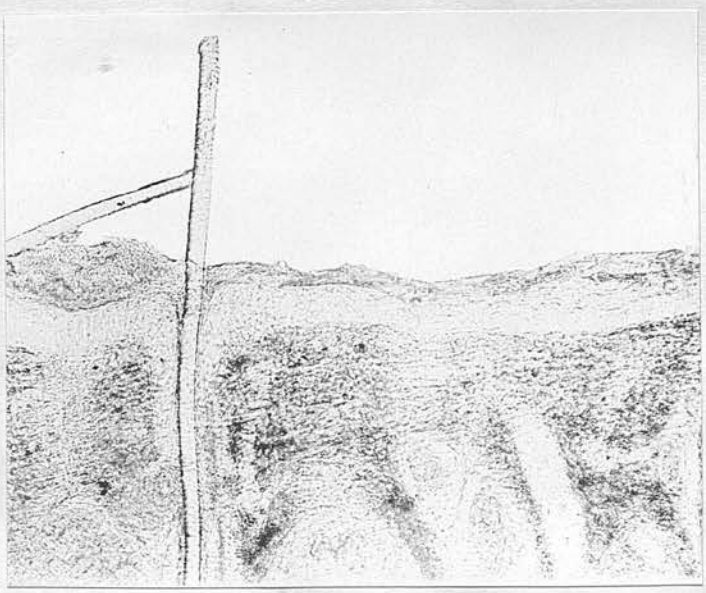
Dominant White

PLATE I



Recessive Brown

Badger face White portion



Dominant White

PLATE I

potentially pigment-producing and which in the untreated state are indistinguishable from any other cells of the Malpighian or basal layer. The dopa-positive cells are mainly confined to the epidermal layers of the skin, the fibre sheath and hair bulbs, but may appear in the dermis. In one instance (Series II, 5, in sections of a skin sample taken from a white area on the belly) dopa-positive melanoblasts were recorded in the dermis. The dendritic form of the dopa-positive cells occurs most frequently in pigmented skin which has been specially stimulated (e.g., by X-rays, sunlight, etc.) but is often seen naturally in fibre sheaths.

The branched fusiform pigmented cells of the dermis which do not give a dopa-positive reaction are chromatophores containing ingested pigment.

The following notes describe the microscopic findings in the various skin sections treated by dopa under identical conditions:-

Series I. Lambs.

1. Brown. Bright brown pigmented fibres; small regular dopa positive cells in epidermis; minute brown granules throughout epidermis; dendritic cells in fibre sheaths. (See Plate I.)

2. Recessive black. Heavily pigmented black fibres; massed dopa-positive and dendritic cells in Malpighian layer/

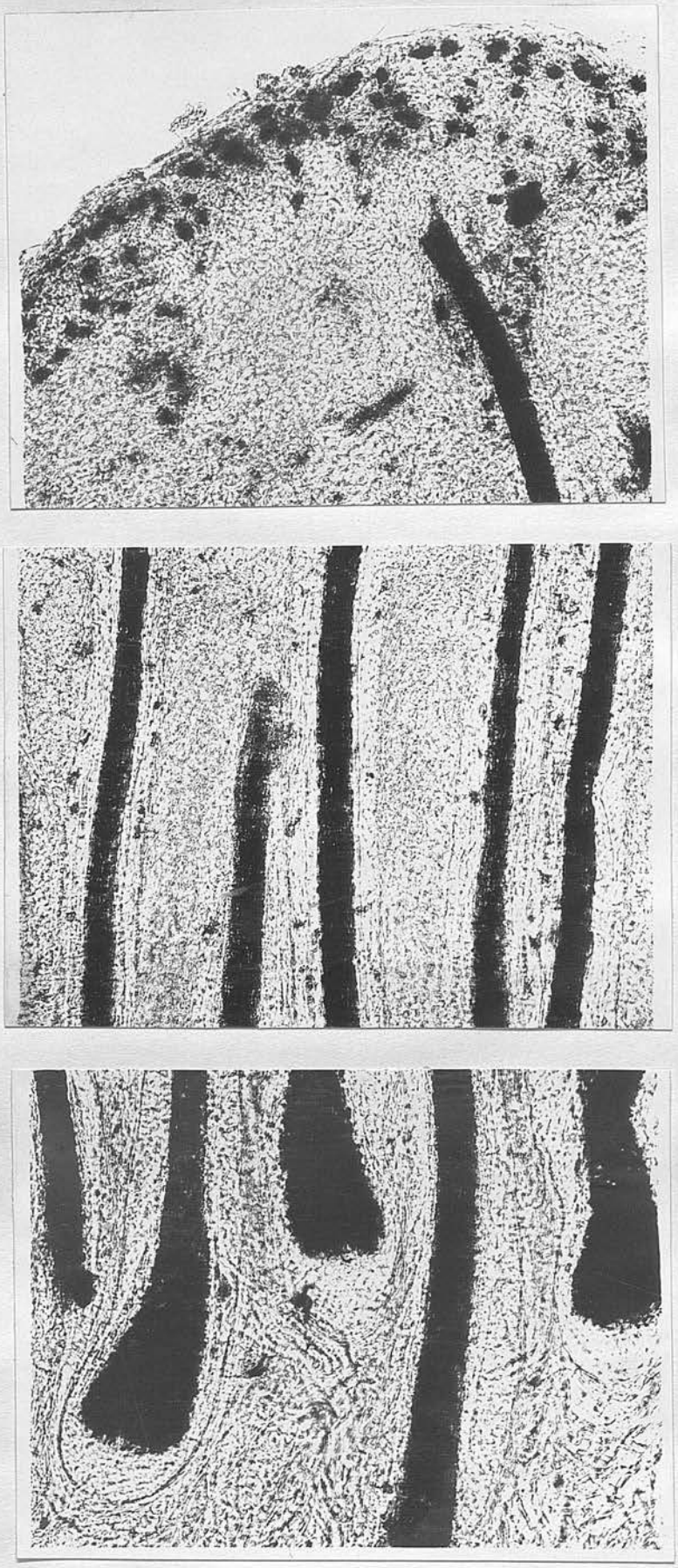


PLATE II Badgerface Sheep - Black Area

layer; brown granular pigment in epidermis; dendritic cells in fibre sheaths. (See Plate III)

3. Badger-face. White portion. Fibres colourless; practically continuous chain of dopa-positive basal cells in the epidermis; deposition of minute granules of brown pigment throughout epidermis; dendritic cells bearing large well marked dendrites. (See Plate I).

4. Badger-face. Black portion. Fibres densely black; many dendritic dopa-positive basal cells; numerous minute granules in epidermis. [see Plate II]

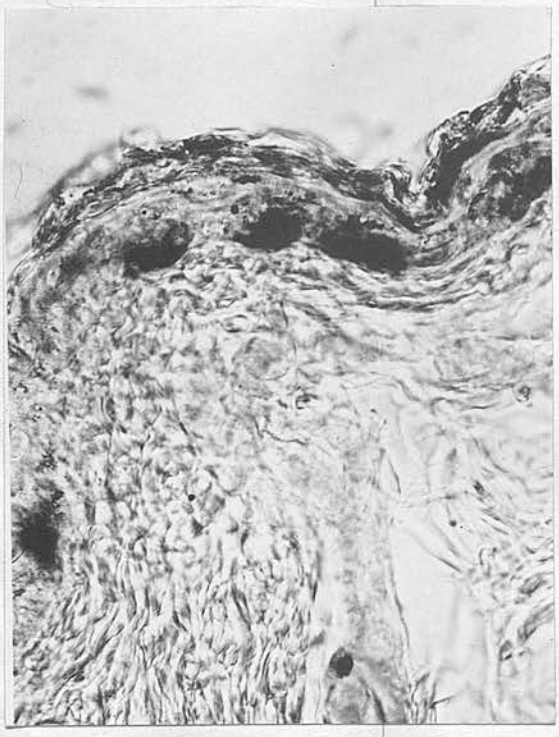
5. Reversed Badger-face. Black portion. Evenly pigmented fibres; numerous clean-cut basal dopa-positive cells; well-defined dendritic cells in certain follicle sheaths.

6. Reversed Badger-face. White portion. Most fibres colourless but a few isolated pigmented ones; numerous well-formed basal dopa-positive cells; pigment production very restricted round certain follicles; dentritic cells very well-defined especially in fibre sheaths.

7. Dominant White. (Spot from black lamb). Complete absence of any reaction. (see Plate I)

8. Dominant Black. Fibres intensely pigmented; many small regularly-shaped dopa-positive cells throughout/

Low Power (i)



High Power of Section a (ii)

High Power (iii)

PLATE III Recessive Black

throughout Malpighian layer and follicle sheaths; isolated dendritic cells in follicle sheaths.

Series II. Adult sheep.

1. Recessive brown. Brown pigment granules in epidermal cells; no basal dopa-positive cells in epidermis; a few dopa-positive cells seen in cross sections of follicle sheaths.

2. Heterozygous white. Sections diffusely stained with yellowish brown; a certain number of dopa-positive basal cells visible.

3. Recessive black. Some heavily pigmented black fibres and others colourless; small dopa-positive basal cells in epidermis and follicle sheaths; bulb cells of colourless fibres exhibit pigment formation.

4. Black, with isolated white spot. Shafts of black fibres very dark; white fibres showing small amount of pigmentation brown pigment in bulbs; a very few minute dopa positive cells in epidermis.

5. Reversed Badger-face. (Banded wool). Bright brown fibres and occasional grey ones; few small dopa-positive cells in epidermis and follicle sheaths; aggregation of melanoblasts in dermis.

6. Dominant white. No reaction, beyond a general diffuse browning.

7. Brown and White area from face of Gritstone x Cheviot. Diffuse brown staining restricted

to/

to epidermis; fibres white with pigment streaks; bulbs very black (i.e. intensive melanin formation); few minute dopa-positive cells in epidermis.

8. Dominant white. No dopa-positive cells and absence of even a slight general dopa reaction.

A comparison between the results obtained with lambs and with adults illustrates the decreasing capacity of the tissues to respond to the dopa reaction. This is correlated with increasing age. In no case was the number of dopa-positive cells in the adult equal to that in the lamb and in the former dendritic cells were never recorded whilst they occur frequently in the lamb. That the decreased dopa reaction was not due to the action of inhibitors was clearly shown by the presence of heavily pigmented fibres in skin sections which gave only a weak reaction. This indicated that in life oxidase production was restricted to the cells of the bulb of the fibre. Apparently restriction in the production of dopa oxidase is a natural accompaniment of age, and for this reason sections from lambs' skins are the more suitable for use in pigment studies. The presence of minute pigment granules throughout the epidermal layers in the lambskin sections is also indicative of the greater melanogenic power of the young skin. This pigment disappears in the form of dust during the process/

process of desquamation characteristic of all epidermal tissues. (Plate III ii).

The appearance of dopa-positive cells in white portions of sections from badger-face skins would seem to indicate an inability on the part of the cells of the white area to make use of dopa in circulation, which however respond readily in a passive environment. The absence of any reaction in dominant white skin would indicate the presence of an inhibitor. Confirmation of this hypothesis will be sought when it is practicable to carry out skin extract experiments.

Beyond the differences in disposition and morphology of the dopa-positive basal cells and dendritic cells it is difficult to define the distinction between colours. Recessiveness or dominance probably depends on the relative strength of the oxidase involved, and recent work on plants (Scott Moncrieff) lends colour to this theory.

In order to offer every opportunity for a dopa reaction in the adult skin series, sections were kept in the dopa solution for a much longer period of time than is normally required - thirty-six hours. This did not in any way influence the results. Small quantities of H_2O_2 (1:100) were added to certain sections but again the reaction was not affected. Other sections in order to destroy possible inhibiting enzymes/

enzymes were boiled before staining, but there was no apparent change in the resultant reaction.

In series 1 (Lamb skins) only No. 8, the dominant black sections were exposed to additional treatment. Neither after boiling nor on the addition of H_2O_2 was there any change in the reaction. This experiment requires further confirmation and extension for the result was not in accordance with that expected and quoted by other workers.

IIb. ii. Experimental in vitro Standardisation of the Reaction.

The variable results after using the dopa reaction indicated the advisability of defining the factors controlling the reaction and accordingly a series of in vitro experiments was planned. The selection of suitable experimental substitutes was necessarily very restricted, and for this reason admittedly not beyond criticism. It is known that light, temperature and pH variation influence the dopa reaction but the extent of such influence has never been determined.

The following is an outline account of the preliminary experiments in this series.

The following reagents were employed except when otherwise stated:-

Dopa (dioxyphenylalanine)

1 mg. per cc. in pure distilled H_2O. (substrate).

Hydrogen Peroxide. (Oxidase)

1 in 200 solution.

Phosphate buffer solutions.

Adjusted to between pH 7.3 - 7.4.

Gelatin.

2 per cent solution in pure water was originally used but in the block experiments a 4 per cent was employed. Little differences were noted in the results. Upon the addition of an equal volume of the dopa solution these concentrations were halved.

Distilled water.

All the reagents were made up in distilled water specially prepared after the method described by Percival and Stewart in Jena glass apparatus.

Toluol.

This was added to the surface of the contents of most of the tubes to prevent the action of atmospheric oxygen.

The method of experimentation by which the pH as well as the concentration of dopa was varied is described by Kermack and Voge.

The use of gelatin in the experiments was for simplification in observation of the reactions. The practice followed in setting up the experiments was to/

to place 2 cc.s liquid gelatin solution in each test tube. To this, the required quantity of dopa solution was added, and as soon as the gelatin had set the peroxide was poured over the surface. Toluol was then added and the tubes were plugged with cotton wool. Readings were taken at definite intervals, the first at the end of the twenty-fifth hour.

Every experiment was suitably controlled and (77) Ridgway's "Nomenclature of Colours" provided the colour standards for comparison and his terminology is used throughout.

Experiment 1.

The effect of atmospheric oxygen upon dopa solutions of varying concentration.

(a) In water
(b) In gelatin.

Dopa concentration	-+	+	++	++++
a)	1	2	3	4
b)	1	2	3	4

At the end of the fourth hour 3a and 3b remained colourless whilst the remaining tubes registered a pinkish buff tinge.

At the end of the twenty-fourth hour a progressive vinaceous buff coloration was recorded for series (a) and an olive coloration for series (b).

Forty-eight hours later the reaction had proceeded still further and series (a) differed little from series/

series (b) save that 4a gave a seal brown coloration whilst in 4b the colour verged towards slate grey.

Conclusion. Dopa solutions oxidise spontaneously in the presence of atmospheric oxygen. The use of gelatin does not vitiate the results.

Experiment II.

The effect of (a) acid, (b) alkali, on the dopa reaction.

A few drops of concentrated Hydrochloric Acid were added to the normal dopa solution and no reaction was observed, whilst a black coloration appeared in the tube containing dopa to which a similar amount of concentrated alkali had been added. The addition of alkali to the tube containing the acid solution stimulated the dopa reaction and a black coloration resulted.

Conclusion. Excess acid inhibits the dopa reaction; excess alkali greatly increases it.

Experiment III.

The effect of varying concentrations of H_2O_2 on the dopa reaction* Concentrated solutions of H_2O_2 completely inhibited the reaction whilst dilute solutions barely initiated it. The optimum concentration appeared to 1:200.

Experiment IV.

The effect of variation in surface area on the

Dopa/

* H_2O_2 Solutions 1:20 to 1:1000 were used.

Dopa reaction, volume being constant. Tubes of increasing diameters were used to contain the reagents. It was found that the greater the area exposed to the action of the oxidase the quicker did the reaction take place.

Experiment V.

The effect of temperature on the Dopa reaction.

Dopa concentration, hydrogen peroxide (volume and concentration) each constant.

Excessive cold, i.e., 0°C, completely inhibited the reaction, whilst heat (55°C) increased the rapidity with which it took place. Tubes which had been exposed to excessive cold reacted satisfactorily when subsequently heated.

Two further experiments VI and VII were carried out on the block system.

Experiment VI.

The effect of variation of pH and Dopa concentration on the production of pigment, the concentration and volume of hydrogen peroxide being constant.

Experiment VII.

The effect of variation of Dopa concentration and volume of hydrogen peroxide on the Dopa reaction. The concentration and pH of hydrogen peroxide being constant. The results are tabulated on charts and illustrated by means of graphs.

Summary/

Summary of observations.

1. Pigmentation increases with concentration of dopa regardless of volume or source of oxygen.

2. Depth of pigmentation appears to be inversely proportional to the volume of peroxide present.

3. Pigmentation alters profoundly with pH, the optimum being found at about 7.4.

4. Hydrogen peroxide disintegration liberates oxygen. it is interesting to note that in general the deeper the pigment the less gas is liberated and it would thus appear that it passes directly into the nascent state from the peroxide into combination with the dopa.

5. In no case was pigmentation produced in the absence of dopa.

6. No reaction takes place in a dopa solution in the absence of oxygen.

7. Temperature profoundly affects the reaction.* Intense cold inhibits pigment formation; heat accelerates its production. Cold followed by heat gives a definite colour reaction; cold after exposure to heat inhibits further reaction, and since the tubes were in darkness during the test it follows that light is not necessary for the reaction.

8. Excess alkali in a dopa solution intensifies pigment formation whilst excess acid completely inhibits it. The addition of alkali to an acid solution produces pigmentation.

In vitro experimentation thus definitely

indicates/

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*Footnote:-

This is of great practical importance in working with skin sections, since samples of fresh skin may be stored in the refrigerator over long periods of time without in any way impairing a subsequent dopa treatment. Sections have been stored in a Frigidaire at $0^{\circ}C$ for two weeks and have still given a normal reaction.

indicates the great influence which environment displays in affecting pigment production, and it seems legitimate to attempt an application of the same principle *in vivo*, where the potentialities of the pigmentary system with its production of melanin may be explored. If it could be shown that varying degrees of pigmentation could be correlated with differential levels of metabolism in the same or different animals a further stage in our knowledge of the physiology of pigment formation would be reached.

The inhibition of the reaction under conditions of low temperature is of interest in that it may throw some light upon the hiberalbic condition of certain animals (See page 135) during the winter months.

One further observation appears of importance. By reference to the charts, pages 39-41, it will be seen that almost any colour of the complete range from Black to Straw-colour could be produced by the necessary adjustments. Colours so closely imitating the colours of the coats of animals as to be indistinguishable from them can be produced *in vitro*, by the use of the same reagents under differing "environmental" (using the word in a chemical sense) conditions; consequently it does not appear to be profitable to postulate the existence/

existence of differing colour pigments; melanin in suitable modification is more probably alone responsible.

Details of Expts VI and VII follow overleaf

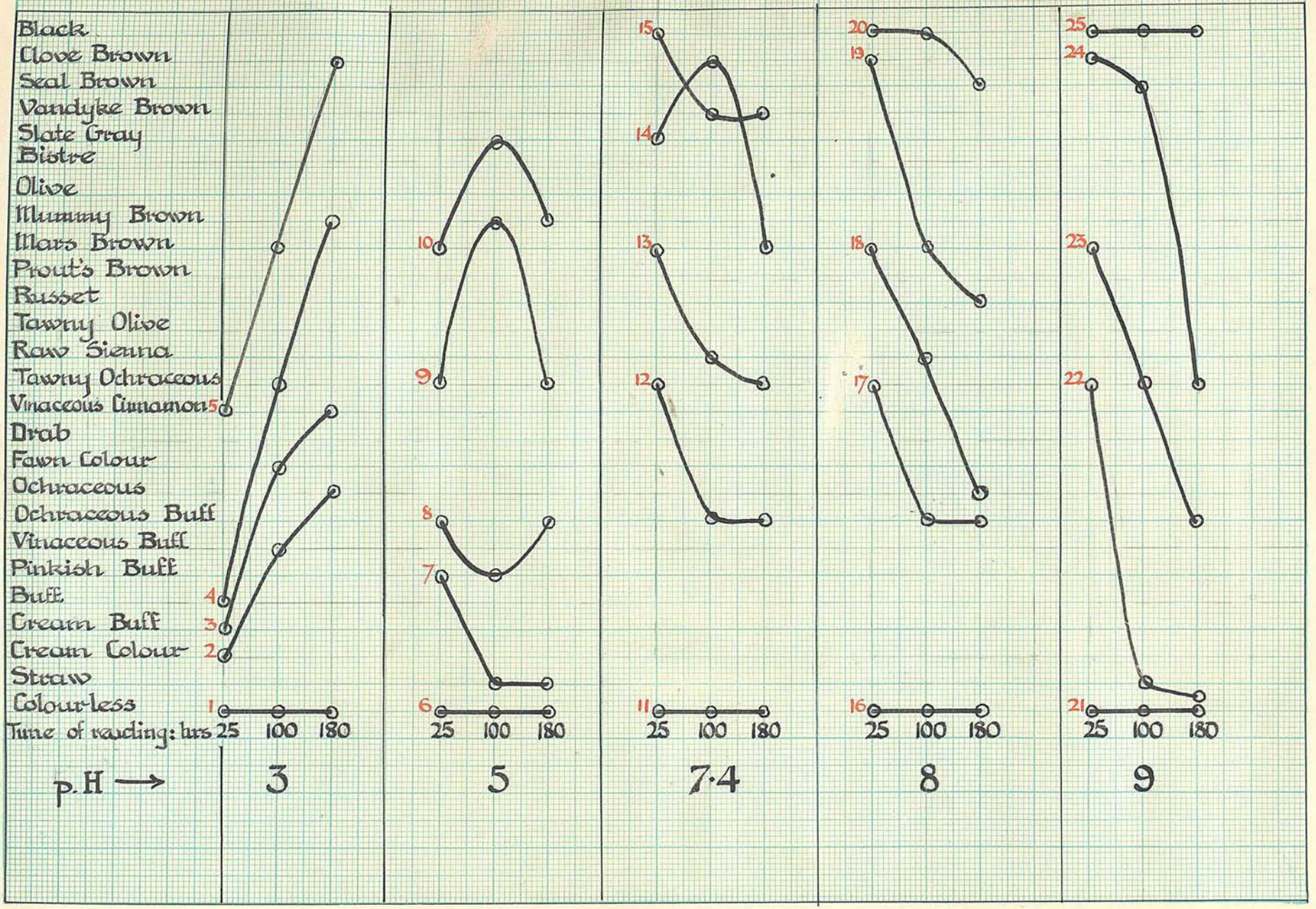
Graph I has been reversed in binding; it should face Chart I

—GRAPH I—

Exp VI

To show the effect of variation of p.H. and Dopa concentration on Pigment production

A



E X P E R I M E N T VI.

Block A.

Effect of variation of pH and Dopa concentrations on the production of Pigment.
The concentration and volume of Hydrogen Peroxide and the volume of Dopa are kept constant.

pH	Time of Reading	Concentration of DOPA -	+-	+	++	++++
3	25 Hrs.	Colourless	Cream colour	Cream buff	Buff	Vinaceous cinnamon
	100 "	1 "	2 Vinaceous buff	3 Fawn colour	4 Tawny ochraceous	5 Mars brown
	180 "	"	Ochraceous "	Vinaceous cinnamon	Mummy brown	Clove brown
5	25 "	"	Pinkish buff	Ochraceous buff	Tawny ochraceous	Mars brown
	100 "	6 "	7 Straw	8 Pinkish buff	9 Mummy brown	10 Slate grey
	180 "	"	"	Ochraceous buff	Tawny ochraceous	Mummy brown
7.4	25 "	"	Tawny ochraceous	Mars brown	Slate grey	Black
	100 "	11 "	12 Ochraceous buff	13 Slate grey	14 Clove brown	15 Vandyke brown
	180 "	"	"	Tawny ochraceous	Mars brown	"
8.0	25 "	"	Tawny ochraceous	Mars brown	Clove brown	Black
	100 "	16 "	17 Ochraceous buff	18 Raw sienna	19 Mars brown	20 Black
	180 "	"	"	Vinaceous buff	Russet	Seal brown
9.0	25 "	"	Tawny ochraceous	Mars brown	Clove brown	Black
	100 "	21 "	22 Straw	23 Tawny ochraceous	24 Seal brown	25 "
	180 "	"	Pale Straw (-)	Ochraceous buff	Tawny ochraceous	"

(Tube numbers in red)

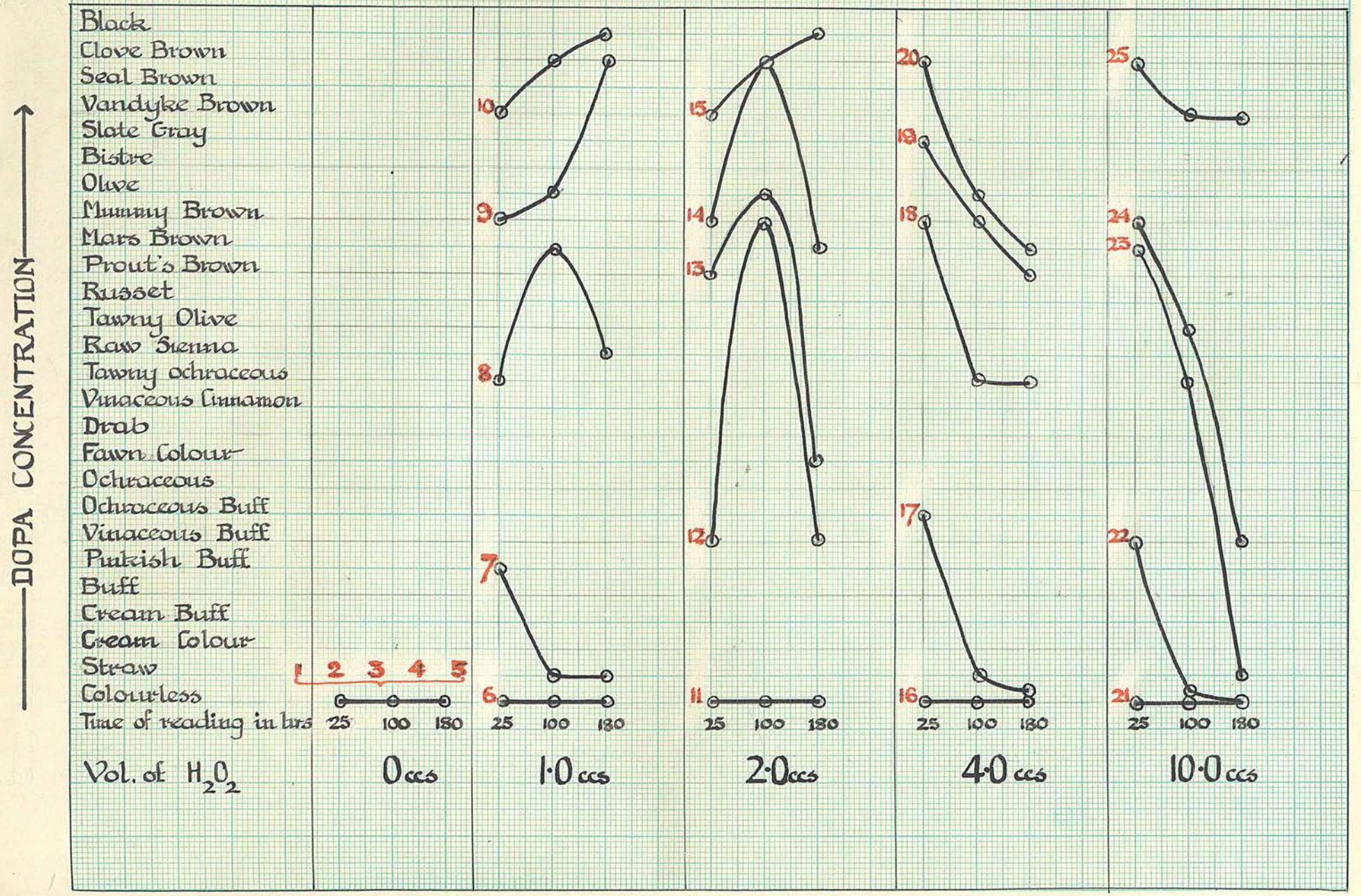
CHART I

—GRAPH 2—

Exp VII

To show the effect of variation of Dopa concentration and volume of Hydrogen Peroxide on Pigment production

B



E X P E R I M E N T VII.

Block B.

Effect of variation of Dopa concentration and volume of Hydrogen Peroxide on Pigment production. The concentration of Hydrogen Peroxide and pH are kept constant and the volume of Dopa does not vary. Tube numbers in red.

Volume of H_2O_2	Time of Reading	Concentration of DOPA –	+–	+	++	++++
0 c.c.	25 Hrs.	Colourless	Colourless	Colourless	Colourless	Colourless
	100 "	1 "	2 "	3 "	4 "	5 "
	180 "	"	"	"	"	"
1.0 c.c.	25 "	"	Pinkish buff	Tawny ochraceous	Mummy brown	Vandyke brown
	100 "	6 "	7 Straw	8 Mars brown	9 Olive	10 Clove brown
	180 "	"	"	Raw sienna	Clove brown	Black
2.0 c.c.	25 "	"	Vinaceous buff	Prouts brown	Mummy brown	Clove brown
	100 "	11 "	12 Mummy brown	13 Olive	14 Clove brown	15 Black
	180 "	"	Vinaceous buff	Fawn colour	Mars brown	Vandyke brown
4.0 c.c.	25 "	"	Ochraceous buff	Mummy brown	Slate grey	Clove brown
	100 "	16 "	17 Straw	18 Tawny ochraceous	19 Mummy brown	20 Olive
	180 "	"	Paler straw	" "	Tawny olive	Mars brown
10.0 c.c.	25 "	"	Vinaceous buff	Mars brown	Mummy brown	Clove brown
	100 "	21 "	22 Palest straw	23 Tawny ochraceous	24 Tawny olive	25 Vandyke brown
	180 "	"	Colourless	Straw	Vinaceous buff	Vandyke brown

CHART 2

IIc. THE BEHAVIOUR OF PIGMENTED AND COLOURLESS SKIN AFTER TRANSPLANTATION TO A DIFFERENT ENVIRONMENT.

During the course of this study it became important to determine whether colour expression in the cutaneous system could be induced to change after transplantation to a new body environment.

Mice were adopted as experimental material and such was the variation in the post-operative reaction of the individuals involved that to ensure retention of grafts several methods of skin transplantation had to be used. In the initial stages three types of grafts were made.

1. Autotransplants, in which skin was transferred from one area to another in the same individual.

2. Syngenesiotransplants, where host and donor were nearly related individuals - litter mates, etc.

3. Homoiotransplants, where host and donor were not related.

It soon became evident that except in the case of autotransplants (where age is a negligible factor), the age of the host and of the donor, especially of the latter were of great importance, and in all subsequent work, age has been of a primary consideration. It was found that the optimum age of the donor lay between four and eight days, whilst that of the host could, with certain exceptions, range/

range between six weeks and three months. With this marked discrepancy in age between the two it was not considered necessary to take into account a difference in sex although in autotransplants where for preference sexually mature mice are used, sex does influence the results.

Albino, self-coloured and piebald individuals were used.

COLOUR CHARACTERIZATION	SKIN	HAIR
Group I.		
Albino	Pink	Colourless
Group II.		
Self-coloured) Black;Grey;Brown) Fawn)	a) Pigmented	a) Pigmented
	b) Pink	b) Pigmented
	c) Irregularly pigmented	c) Pigmented
Group III.		
Piebald) Black/white) Grey/white) Fawn/white) Choc./white)	a) Exact colour correlation between skin and hair	
	b) Pink	b) Piebald patterning
	c) Irregularly pigmented	c) Piebald patterning

Unfortunately, in the available stock all piebald and self-coloured mice showed to a greater or lesser extent the "Schultz reaction". This reaction, which has been established as one of the important factors in animal pigmentation, was first demonstrated by Schultz (90) (See Fig. 6). It is simply that cold increases pigmentation, heat reduces it. He based his/

his description and definition of the phenomenon on his experiments with the Himalayan rabbit, but quoted various other animals where the reaction might also be observed. Therefore, according to Schultz our mice may be said to have the same genetic colour constitution as the Himalayan rabbit, since, whilst heat reduces the quantity of pigment produced, cold evokes a greater response; at the same time this does not imply that our mice have dark extremities, as in the Himalayan rabbit. The importance of this reaction cannot be overestimated in any work on pigment since in animals with the genetic colour constitution of the Himalayan rabbit, any local increase or decrease in the temperature will affect the skin pigmentation. When skin grafts are being studied in their relation to skin pigmentation, it is obvious that the Schultz reaction introduces a complicating factor which makes it extremely difficult to estimate the influence of the transplantation operation itself. The fact that different individuals exhibit the reaction in highly varying degrees is another point to be considered, and up till the present no method has been evolved by which it is possible to eliminate or control this variation.

With such an infinitely varying product as pigment it is impossible to define its behaviour within/

within narrow limits and in the experiments about to be described it was not practicable to standardise the available material beyond the initial selection. This implies that in Group II, b) and c) may merge into each other, whilst in Group III, a) and b) may come to resemble c). The exposure of the animals to a lower temperature is an inevitable accompaniment of operative work, since as a preliminary, the hair covering must first be removed from the operative field thus lowering the temperature of that special area of the skin. As yet, no method of bandaging has been devised which would prevent heat loss from the denuded area before and after grafting and which might possibly inhibit a Schultz reaction.

Since skin injury also influences pigment production it has been necessary to consider carefully the method by which the hair can best be removed.

Both physiological and mechanical means have been employed and whilst neither is ideal the latter is technically simpler and very much quicker.

On account of their irritant powers and general disturbance it was not thought advisable to use local depilatories.

With shaving, small scarifications and a certain amount of bruising are unavoidable, and it is in the region/

region of these incisions that pigment is rapidly produced. Since, however, they heal easily (in two or three days' time), and the accompanying pigment disappears shortly afterwards, their presence need not be an unduly disturbing factor provided an operation is not carried out until the skin is again healed.

Thallium acetate given per os is an agent which induces shedding of hair but its use is restricted to young mice since its administration in effective quantities to mature mice always produces fatal toxic results. A further disadvantage lies in the fact that practically all the body hair is shed exposing a far greater surface than necessary and finally, the period of time, three weeks and more, which must elapse before the hair is replaced, greatly prolongs the experiment and offers every opportunity for unmeasured complications. The use of thallium acetate in skin transplantation experiments is however to be explored further.

Several types of skin transplantation have been tried; they may be grouped under four main heads.

I. Free Grafts
   a) Small
   b) Large

II. Implant Grafts
   a) Free
   b) Attached
   c) Open
   d) Slot grafts

III. Follicle Grafts

IV. Pedicle Grafts

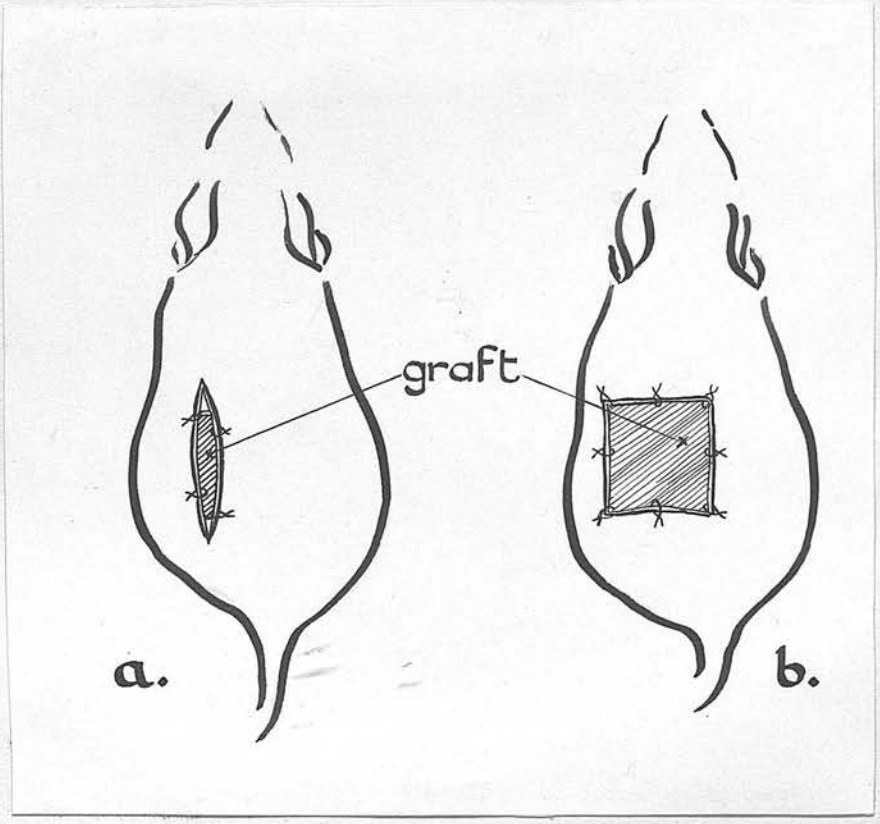


FIG. 1. FREE GRAFTS. (a) Small. (b) Large.

In I and II, Autotransplantation, Syngenesiotransplantation and Homoiotransplantation have been carried out. Homoiotransplants have been made in Group III, whilst in Group IV all the grafts are necessarily Autotransplants.

Throughout the experiments surgical asepsis had to be observed and in the case of syngenesio-and homoiotransplants its importance cannot be over-estimated.

In the mouse it was never possible to maintain indefinitely a homoiotransplant and for this reason much more attention has been paid to auto-and syngenesiotransplantation.

I. Free Grafts.

a) Fifteen small free grafts were made, using eleven coloured and four white adult mice as hosts, and young unrelated coloured and white mice as donors. The practice followed was to graft coloured skin on to colourless individuals and colourless skin on to coloured mice. In no case did the graft exceed 1.5 cms. in length and .5 cms. in breadth. It was always implanted in the back posterior to the ribs and slightly to one side of the spine. (Fig. 1a). Fine silk thread was used for fixing the graft in situ, and the fewer the sutures the less was the injury to the graft. In three cases the graft was sloughed on the third day after operation, in nine, on the seventh, and/

and in the remaining three on the tenth day. There was never any change in the colour of the graft. In the coloured mice sloughing of the graft was always accompanied by pigment formation along the margins of the grafted area and this persisted till the hair was regenerated. The scars disappeared very rapidly without leaving any visible trace and this suggested that a larger graft necessitating a larger incision in the skin of the host might have more chance of survival.

b) The large grafts were usually not more than 2.5 cms. x 1.5 cms. and as in smaller grafts were transplanted on to the back of the hosts. (Fig. 1b.) Numerous grafts were made, but in no case was there a perfect "take". The grafts persisted for a period varying from fifteen to twenty-seven days, the latter being the extreme length of time, but beyond that they did not differ from the small grafts. The replacing skin in the hosts, however, never attained normality and the growth of hair on such areas was always sparse. No colour change took place in the graft and the growth of the hair on it was greatly retarded.

Homoiotransplantation having proved a failure, syngenesiotransplantation of free grafts was next attempted. The best results were obtained when very young mice, four-day-old litter mates, were used, both as/

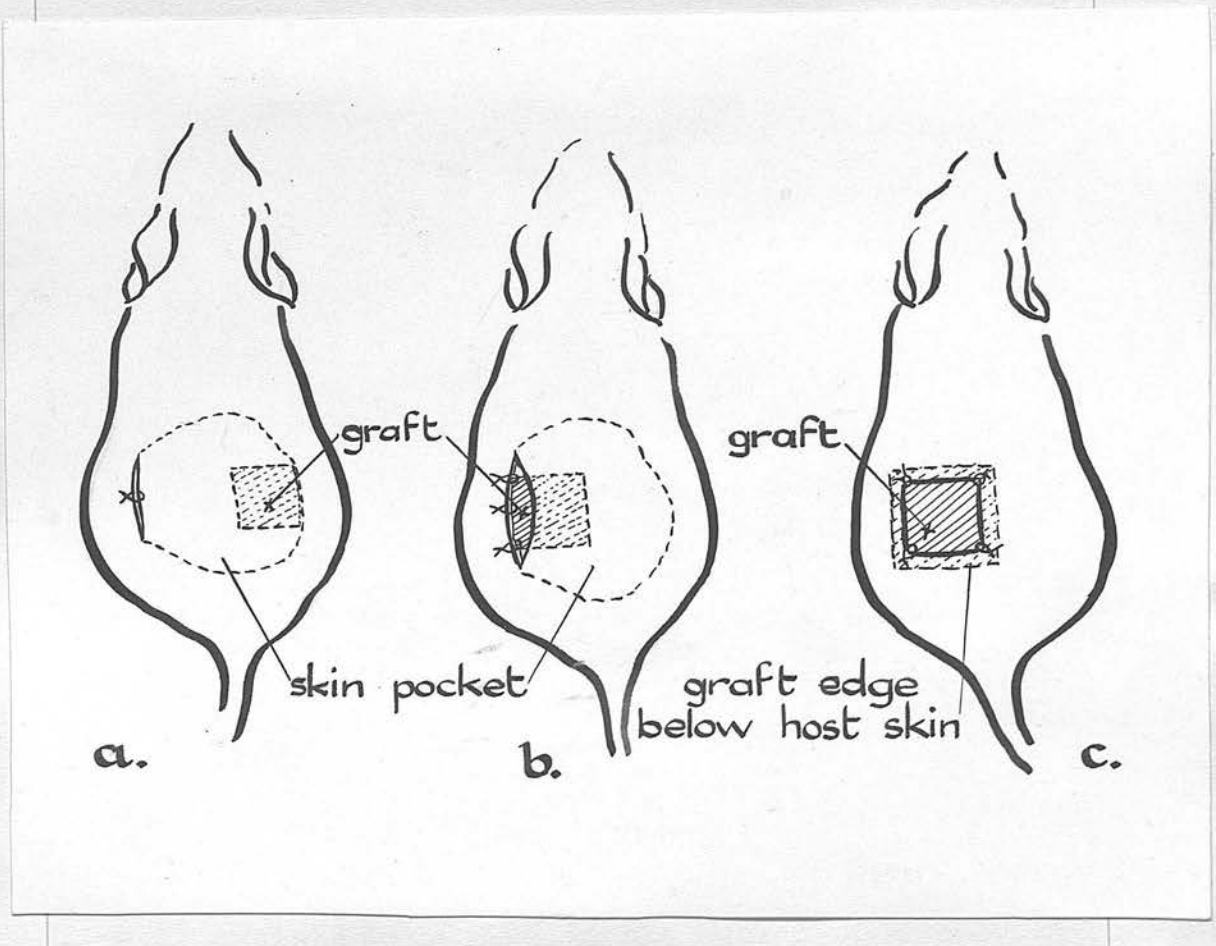


FIG. 2. IMPLANT GRAFTS. (a) Free. (b) Attached. (c) Open.

as hosts and donors, and under these conditions it was possible to transplant skin successfully. One very great drawback of this method is that at four days old the skin colour and pattern, hair type, etc., are not well and clearly defined and it is impossible to estimate to what extent the graft either retains its individuality, or adopts that of the host. The survival of the syngenesiografts is frequently a matter of chance for often the mother destroys all individuals so grafted. This is in strange contrast to the tolerance which the mother mouse exhibits when the grafts are from sources other than her own offspring. A further exploitation of this method is being carried out and later it is proposed to adopt the "Siamese twin" technique —— parabiosis.

II Implant Grafts.

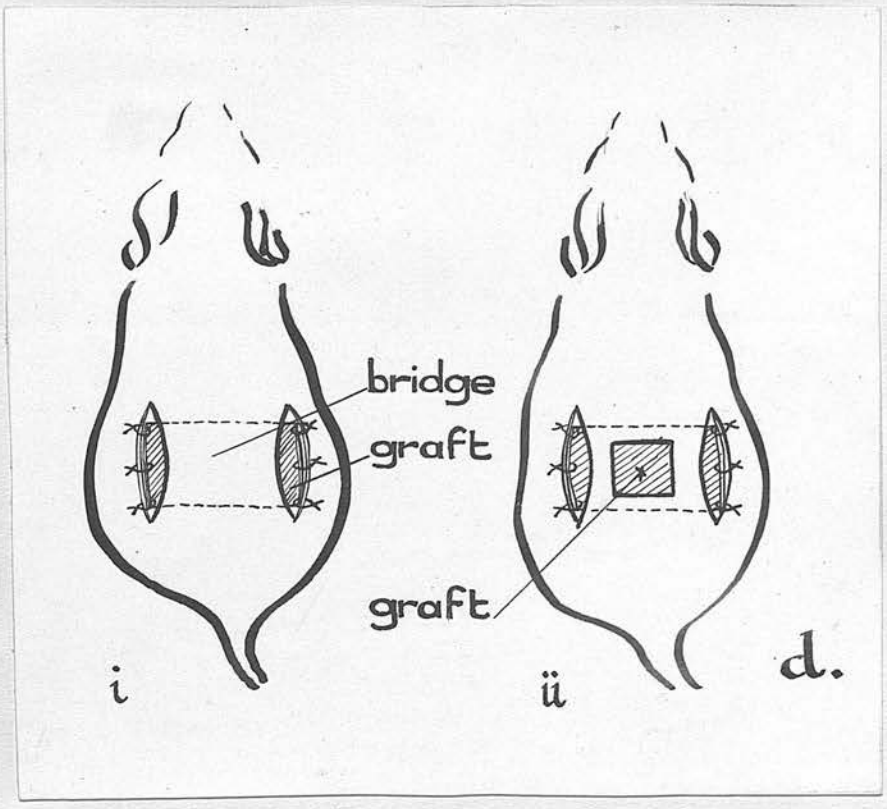
a) Free Implant. Adult mice were used as hosts. A small incision was made in the back of the host, and the underlying connective tissue cut so that a large pocket was formed. Into this a piece of skin from a four-day-old mouseling was placed and the incision then stitched up. (Fig. 2a). As a rule the implant travelled to the deepest part of the pocket. Four days after the implantation, the skin immediately above the implant was removed and the graft exposed.

Attachment/

Attachment by the lower surface had occurred and the grafts were always healthy. If however the covering skin was not removed till the seventh day after the operation, the graft was found to be encapsulated by connective tissue, —— part of the process of normal obliterative repair of a subcutaneous cavity. At this stage the implanted graft had already begun to degenerate. If, after exposure of the implant graft, the graft was not sutured in situ, it was either eaten out immediately or sloughed within two days; stitching, whilst it did not induce the graft to take, prolonged the duration of its retention by the host.

When the host was coloured and the graft white, no colour change took place in the transplant, but when the host was an albino and the graft pigmented, the effect of implantation was a very definite bleaching of the graft. This however was not due to any influence of the host but to a reduction in the amount of available oxygen, since,whenever the covering host's skin was removed, the graft regained its original colour.

b) Attached Implants. These differed from the free implants in that one side of the graft was stitched to the farther edge of the incision and the incision itself was left gaping so that a small portion/



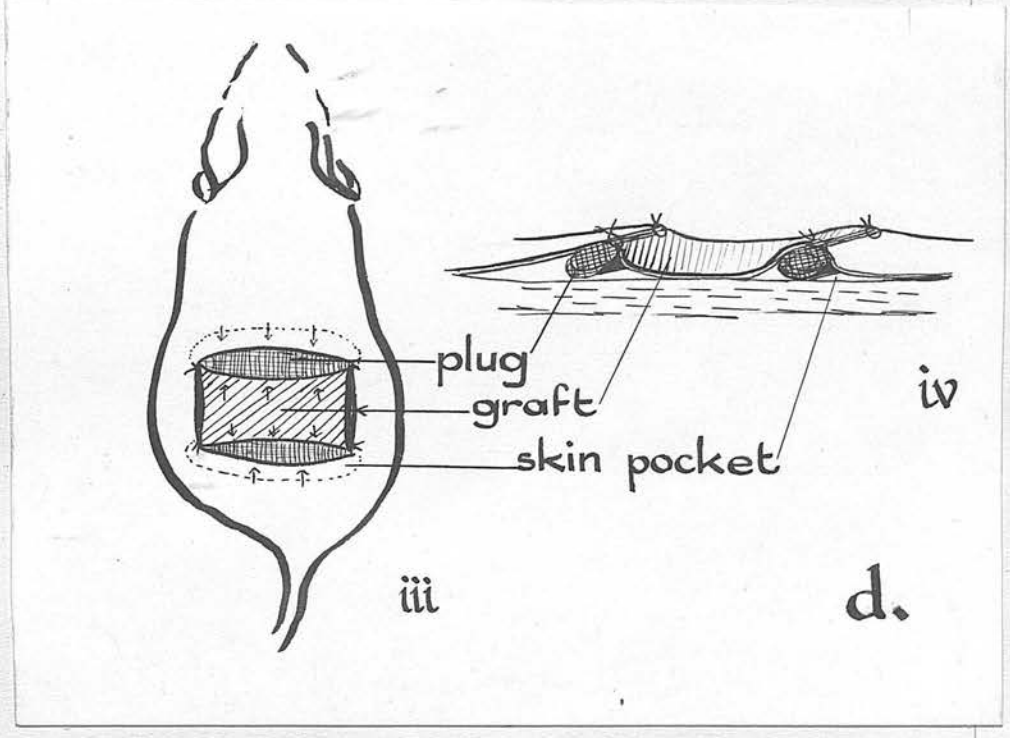


FIG. 3. IMPLANT GRAFTS: (d) Slot grafts. i. Insertion of graft below bridge of host skin and method of suturing. ii. Removal of portion of bridge skin two days after operation. iii. Complete removal of bridge skin and insertion of plugs five days after operation. iv. Diagram to illustrate method of plugging.

portion of the graft was exposed. (Fig. 2b). Only a very small percentage of these grafts was maintained beyond two days since the operated animals almost invariably ate out the graft within the first forty-eight hours. In a few cases the graft persisted for thirteen days and during this time the host skin covering gradually dried and withered, and about the twelfth day was thrown off exposing an almost equally shrivelled graft. The sloughing of the graft followed soon after leaving a bare but completely healed patch of new host skin.

c) Open Implants. In these, about a square cm. of skin was removed from the back of the host, and the underlying connective tissue cut back from the edges. A graft of 1.5 cms. square was then placed in the opening and the four edges tucked in under the host skin. The graft was then stitched in place. (Fig. 2c). The retention of the graft never exceeded twelve days and was often less. As in the attached implants the host skin covering the edges of the graft began to wither, new skin being formed under the graft. Again the bleaching of the edges of the coloured grafts was observed, but there was no change in the white ones.

d) Slot Grafts. The slot graft is an extension of/

of the open implant and, of all methods of free grafting, is the most satisfactory. Two incisions 2.5 cms. apart and 2 cms. in length are made on the back of the host, care being taken not to sever any of the larger blood-vessels. This is a precaution which must be observed. The skin between the slots is dissected from the subcutaneous tissue to form a bridge so that there is a free space between incisions. A piece of skin slightly less in area than the host's skin between the incisions, is then tucked through one of the incisions and straightened out. The margin of the graft is stitched to the outer edge of one incision and the opposite edge to the outer margin of the other incision. The incisions themselves are left gaping. (Fig. 3(i)). Two days later a square centimetre of skin is removed from the host skin bridging the graft. (Fig.3(ii)) and then as soon as the host skin has almost withered back to the still covered edges of the graft, the host skin is cut away to just beyond the unstitched graft edges and plugged with lint. (Fig.3iii). This withering usually occurs about the fifth day but may happen sooner. Should the critical moment for removal and plugging of the host skin be missed, nothing will save the graft, for once new skin has begun to colonise the tissue below the transplant, the latter/

latter is quickly under-run and its attachment lost. As in former instances, the coloured graft loses its colour and the white remains unchanged, irrespective of the particular colour of the host.

III. Follicle Grafts.

Grafting of individual follicles is now being attempted, but results to date do not differ from those obtained with grafts of intact skin. It is however a fact that whilst a small implant graft or even a large one perishes fairly quickly, an implant of a few follicles maintains a connection with the host for a period at least as long as the larger implants and longer than the smaller ones.

A small piece of skin from the donor is placed in warm saline or Ringer's solution and the follicles are dissected out. Clumps of seven and eight are at present being used. The skin of the host is then lightly incised, the incisions being parallel, not more than 1 cm. in length and at right angles to the direction of hair growth . No bleeding should occur and the number of incisions should not exceed twelve. After implantation of the follicles, it is advisable to put one stitch in every incision, since although these close rapidly, later movement of the animal after recovery from anaesthesia tends to make them reopen and thus dislodge the follicles. In these grafts, which have/

have so far not persisted beyond twenty-seven days, there have not been any changes in the colour of the individual follicles.

IV. Pedicle Grafts.

Skin transplantation by means of pedicle grafts is always successful and the grafts replace perfectly and permanently the skin removed from the host. With special reference to the behaviour of pigment in such grafts, mainly piebald mice have been used, but for comparison and control similar grafts have been made on both white and self-coloured animals.

An L-shaped area is shaved on the back and lightly swabbed with 70% Alcohol (See Fig. 4). Two longitudinal incisions (AB, CD) 2 cms. apart and 2.5 cms. long are made, and then two further incisions (DE, FG) parallel to each other and at right angles to AB CD (See Fig. 4(i)). They are 2 cms. in length and 1.75 cms. apart. The rectangular piece of skin over the area defined by DEGF is now removed, an incision AC is made, and the skin covering the area enclosed by BACDF is dissected away from the underlying connective tissue. This loosened skin which is to be transplanted is sutured in position over DEGF, BF being the pedicle. Within two hours of operation it is possible to decide whether or not circulation between the graft and/

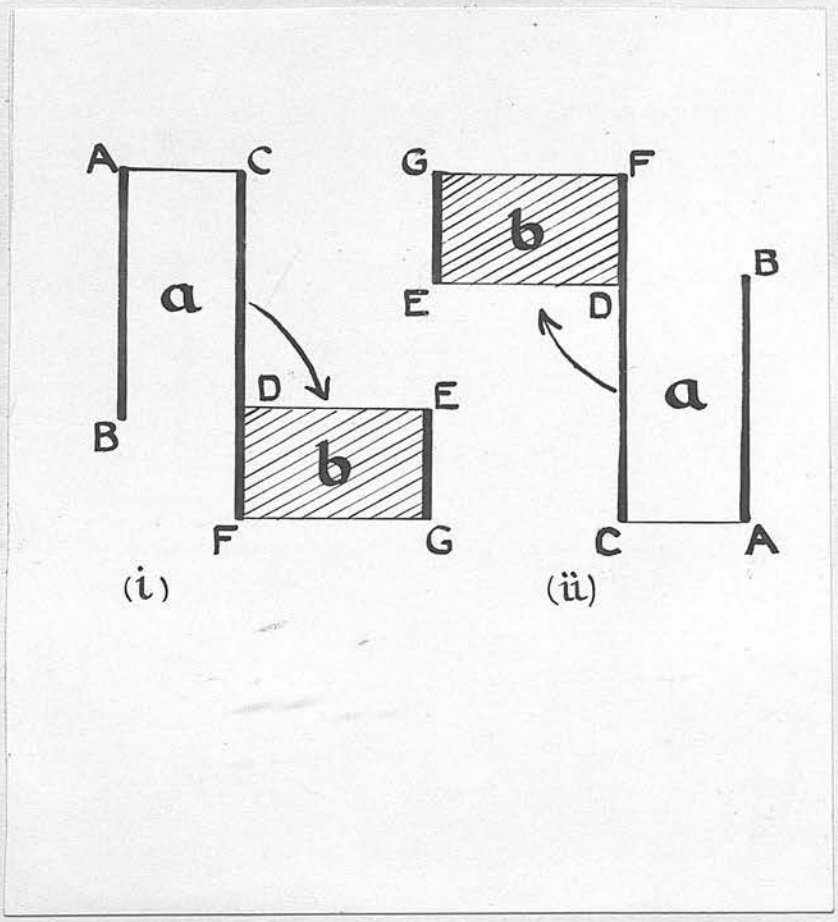


FIG. 4. PEDICLE GRAFTS :(i.) The portion of skin "a" is moved posteriorly and to the right to replace "b". (ii.) The portion of skin "a" is moved anteriorly and to the left to replace "b".

and the surrounding skin has been initiated. After four days, healing by first intention is far advanced and by the sixth the scabs have sloughed. At this stage the pedicle may be severed by removing a strip of skin .25 cms. wide; the raw edges then being stitched together. The progress of the graft after this date is uneventful. The time at which the pedicle is resected is of considerable importance since if it is done within five days after the operation, plugging must be resorted to, or the graft will wither and there will be rapid growth of new skin below it. If however there is no special reason for cutting the pedicle soon after the operation, this should be left for about ten days when the pedicle may be cut with safety. The position of the graft must also be considered, for in postero-anterior transplants it is necessary to allow the pedicle to remain attached for a longer period than in antero-posterior ones. The plugging (cottonwool in lint, soaked in boric acid ___ 2 per cent sol) must be kept damp or the graft withers and is sloughed. Blood clots too, cause the overlying skin to degenerate and care must therefore be taken to avoid them.

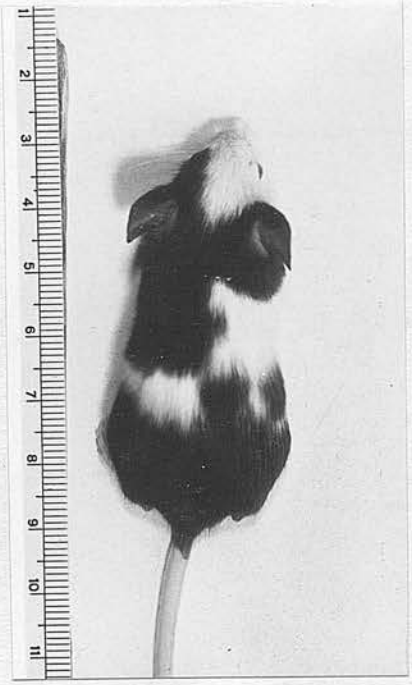
In all, forty pedicle grafts have been made. In the piebald mice, coloured skin was grafted to replace white/

white and vice versa. In those animals where the skin pattern exactly corresponded to the hair coloration this presented no difficulty, but in piebald mice with a wholly pink skin it was necessary before shaving to outline by means of tattooing, the colour pattern of the hair.

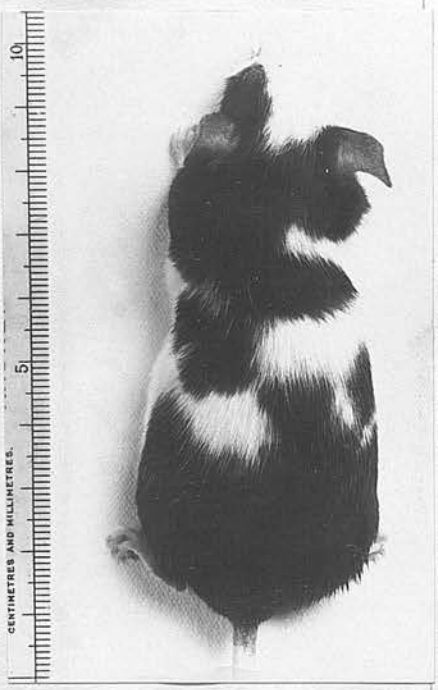
In every case, the grafted skin retained its original colour and produced hair the same colour and type as before, quite independently of its new environment. However, in those cases in which the pigmentation of the skin and that of the hair did not correspond the irregular patches in the grafted skin always disappeared after transplantation, whether in an area producing coloured or white hair.

Effects of Skin Transplantation.

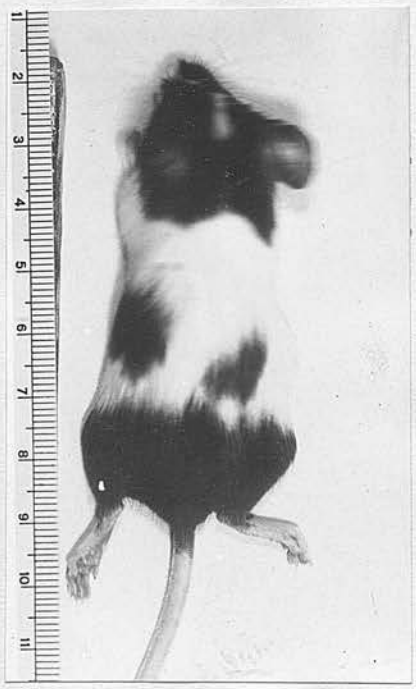
When coloured skin is transplanted to a white area the growth of hair usually regains its original luxuriance but only after the lapse of time. When white skin is transferred to black, regeneration of the hair is rapid. Amongst the coloured hair of a graft growing amidst white hair, however, it is usual to find a certain proportion of light and even colourless fibres, and in one instance a few banded fibres were produced. A flecked appearance often characterises the dark-haired grafts and is due to the lack of pigment in the extreme tip of the fibres. This lack of/



Before Operation



9 Months after Operation



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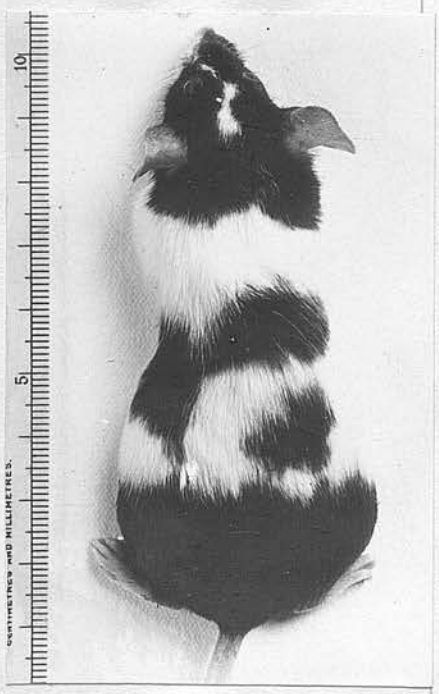
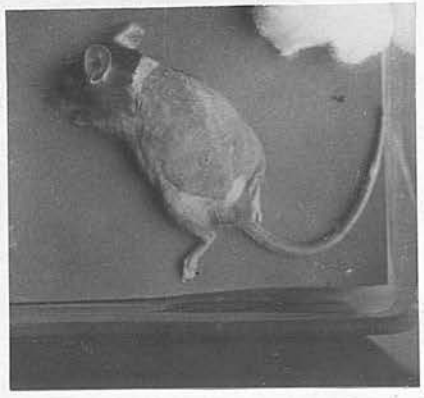


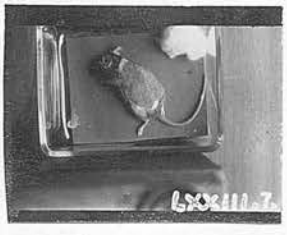
9 Months after Operation

FIG. 5 Photographs of Mice with Pedicle Grafts

Fig. 6. The Schultz reaction in the mouse. White skinned mouse carrying black hair. The mouse was shaved over the darker central dorsal area of the body seen in the photographs. Pigment was deposited in the denuded skin within seven days. When regeneration of hair (coloured) began, the mouse was again shaved to beyond the edges of the original area.

In the photographs the lighter margins around the originally shaved area represent skin where no Schultz reaction has occurred. The darker central area exhibits the Schultz reaction.





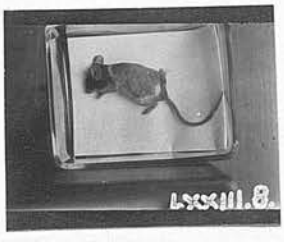


FIG. 6 THE SCHULTZ REACTION IN THE MOUSE

of pigment is a quite usual concomitant of the shock which is produced in the animal at the time of operation. As soon as the effects of the shock disappear, pigment production proceeds normally. However, in certain black-haired individuals the operative shock has been such as to inhibit completely the production of further pigment in a grafted area.

The direction of the hair, especially in pedicle grafts where the skin is seldom in the same relation to the long axis of the body as it was before, sometimes differs from that over the body generally. In one animal, which was grafted on December 9th, 1930, and was killed on January 22nd, 1931, there was over the transplant a luxuriant regeneration of hair which was growing at approximately right angles to that on the rest of the body. Where the hairs of the graft were in contact with the normal body hairs, they exhibited a tendency to alter their direction and to conform with the rest of the hair, whilst in the centre of the graft the original hair direction remained unchanged.

In mice which are grafted at a very early age, six to eight days old, the development of hair on the entire posterior part of the body is, for two or three weeks and sometimes longer, below normal. Grafting has no apparent ill effects on the growth of hair further/

further forwards.

One of the most constant occurrences in the skin after shaving and grafting is patchy regeneration of hair. In all coloured and piebald mice the coloured portions of the skin are always the first to regenerate hair and it would seem that pigment-production is a direct stimulant to follicular activity. In casual skin pigmentation too - that which has no reference to hair colour pattern - the pigmented patches are the first to produce hair. Often the production of pigment is so rapid and so generous that the skin itself is temporarily raised and lumpy and the condition becomes almost pathological. (Coloured wool fibres occasionally manifest the same condition and may be so overcharged with pigment as to present an irregular outline even to the naked eye).

Discussion of Skin Transplantation.

During the last thirty years transplantation of organs and tissues has especially been employed in the study of individuality. There is a somewhat extensive literature on skin transplantation in Amphibians and Birds, and modern surgery has contributed much to our knowledge of skin grafting in Man, but beyond this the skin is a comparatively unstudied tissue.

Regeneration of lost parts is more particularly the prerogative of the lower forms of life, but even in the mammal there still remains one tissue at least which retains/

retains this power, namely, the skin. In the mouse this regenerative property is extraordinarily well preserved and for this reason the successful transplantation of skin is more difficult than that of other less primitive tissues. The very functions of the skin all militate against its ability to adapt itself to an environment other than the original one and especially potent is its immunity to sepsis. This sensitivity of the skin is developed to such a high degree that there is practically no toleration of even sterile foreign bodies. Child (7) has said that "more active protoplasm is more susceptible to extreme conditions and more capable of adjusting itself to less extreme conditions than the less active". This is clearly demonstrated in our experiments. In hair-less mice, skin grafts may be retained for a period exceeding thirty days, whilst in normal haired mice homoiotransplants have not ever been found to be retained beyond the twenty-seventh day, with an average of twenty days only. Surely there is a direct relationship between the physiological activity of the two strains and the degree of tolerance exhibited by the host for the foreign graft. The more active and the constitutionally stronger individuals react far more intensely than the weaker ones and skin replacement/

ment with subsequent sloughing of the transplant, occurs very much more rapidly.

Even in the case of large grafts where the amount of skin removed is great, the regeneration is complete and after the lapse of several months practically perfect in its constitution. This is in striking contrast to what happens in other animals where the power of regeneration is more limited and the production of scar tissue occurs. Histologically, newly developed skin in time tends to become normal and to assume the characters of the tissue which it replaces. Thus, whilst in small grafts scar tissue soon disappears and hair develops rapidly, in the larger ones the bare skin which remains after the sloughing is only gradually re-covered. The replacing skin takes months to develop a normal number of follicles, and indeed the number of follicles per square cm. in replacing skin never equals that of the surrounding undisturbed areas.

In such a study of individuality it is necessary to define that stage at which a comparison of graft and host tissue is valid. It is our opinion that such comparison is legitimate only when the graft is successful, and a successful graft can best be defined as one which persists for a period of not less than two/

two months. In the skin at least this is not too restricted a definition, for eternally persistent grafts are possible. Schöne (89) carried out a large series of skin transplantation operations in the mouse and in a few cases was successful in producing syngenesio grafts which were maintained for a period exceeding five months. Unfortunately he was not concerned with pigment behaviour and his results do not include any observations concerning it. The reason for stressing this point and for determining exactly when a graft ceases to be a foreign tissue and becomes a functioning part of the host, is on account of the great morphological difference which exists between a temporarily tolerated graft and one which has established complete union with its host. In the former case the changes which take place are not those associated with normal development and as time goes on these abnormal changes are increased, culminating in the actual death of the graft itself. During the period that the graft is retained by the host the growth of hair on the graft is always greatly retarded and in many cases completely inhibited. In contrast to this is the continual functioning of the hair follicles of successful grafts even though there is usually an intervening period of latency in follicular activity.

What/

What is it then that ensures the life of the graft? Recent research has failed to establish the existence of blood groups in the mouse but in homoiotransplants it is obvious that incompatibility of body fluid between host and donor is of paramount importance and this is sufficient explanation for the usual failure of such grafts. In the case of syngenesiotransplants and autotransplants this incompatibility does not exist to the same extent and other factors must be taken into account.

Firstly, we must consider the control of the blood supply to the graft, and secondly, the prevention of proliferation of the host's connective tissue below the graft. The clearest demonstration of both of these points is seen in the case of pedicle and slot grafts. In the case of a strong arterial supply with an inadequate venous drainage system (when a large artery is located near the edge of that incision which is proximal to the heart of the host), one of two contingencies may arise. Either capillary or arteriole haemorrhage will occur at the graft edges and effectually interfere with its adventitious blood supply; or, venous congestion of the skin edges will result, with consequent dislocation of the process of vascularisation of the graft. The resultant pronounced stimulus to proliferation of the edges of the/

the host's skin leads to consequent colonisation with newly developing host skin of the tissue lying below the graft. The extension of the sub-graft colonisation results in the sloughing of the graft after a period of time, inversely proportional to the strength of the blood supply of the proximal edge of the incision in the host's skin. To counteract this active proliferation the principle of plugging was adopted. This consists of the interposition of a barrier of lint or of cotton wool between the area of robust proliferation of the host's skin edge, and that of the more delicate graft which the plug protects.

Perhaps the most important point to observe in successful skin transplantation in the mouse is that the union between host and graft shall be established by edge to edge approximation and not by surface contact. A natural extension of the plugging method is the use of some barrier between the lower surface of the graft and the superficial muscular fascia of the host so that connection can be established only between actual skin edges.

In so far as pigmentation is concerned it was found that a non-pigmented skin adapted itself to a new similar environment far more quickly than did a coloured one. A colourless graft, however, transferred to a coloured area took longer to heal than in the first/

first case, but was relatively more rapidly established than was a coloured skin. It would seem from this that there is a definite although not insuperable incompatibility between white and coloured skin and to a lesser extent between coloured and coloured skin.

Loeb stated that in his transplantations of coloured guinea-pig skin to a white area in another individual the homoiotransplant was always unsuccessful but that in the case of autotransplantation the pigmented skin not only healed in, but rapidly began to penetrate the surrounding white skin. It is possible that what appeared to be proliferation and penetration of the coloured skin was really a deposition of traumatic pigment such as often occurs in piebald skins after injury. His statement that in syngenesiotransplants it was possible for coloured skin to be transformed into white was not confirmed in our work with mice. In several instances apparently successful coloured grafts on white hosts disappeared by a gradual process of desquamation, and the skin which then appeared bore only the white hair typical of the host. In a few cases, however, the coloured grafts developed normally and bore a luxuriant growth of dark fibres which equalled in every respect, save colour, that of the/

the host. These coloured fibres were shed about twenty-five days after the operation, leaving a bare patch of coloured skin which, although itself remaining pigmented, no longer produced hair. It is now possible to suggest that this represents a period of latency in follicular activity of the graft. The interesting point is the continued production of pigment in the grafted skin.

In homoiotransplants a coloured graft only exceptionally produces a growth of coloured fibres, and in practically every case the grafted skin remains bare. In contrast, white grafts on coloured mice are successful and maintain their individuality indefinitely. (It has been necessary to distinguish carefully between actual white haired grafts and abnormal production of white host hair which sometimes occurs in a coloured individual after injury). The numbers of scattered white fibres which occur in a coloured mouse after operation is suggestive of more than just a local reaction to shock, and it is somewhat difficult to reconcile their appearance with the increased pigment production which so commonly accompanies injury of the skin.

A negative secondary pigmentation sometimes occurs in the white skin of a graft bearing coloured fibres and/

and occasional bleaching is seen in the skin of the coloured grafts, but one cannot disregard the possibility that these reactions might have occurred under circumstances other than transplantation (e.g. shaving, injury, etc.) in the original environment, and that therefore they cannot be said to be due to the influence of the host. Melanin is produced only when the pigmentary system is complete and there is uninterrupted interaction between the catalytic enzymes and the basic chromogen. This explains why coloured skin maintains its pigmentation when transplanted to a recessive white environment and also why a white graft on a coloured host remains colourless. Further, it is reasonable to postulate that pigmentation of a coloured skin in a dominant white environment might be inhibited but it is possible that it would continue unaltered. An analysis of the differentiation processes of foetal skin before the macroscopic appearance of pigment would possibly indicate the nature of the catalytic enzymes and the factors controlling their production or inhibition. Such work, foetal mammalian skin grafts on the chorio-allantoic membrane of the chick, is now in progress.

IIIa. COLOUR IN SHEEP.

Opinion is divided as to the importance of colour in sheep but as the science of breeding has developed so has it come to be realised that it is a problem which demands a solution. Superficially, wool is either coloured or colourless. The separation into these two great classes appears to be simple but closer examination reveals the fact that every coloured wool contains white fibres, just as coloured fibres are found in every white wool.

Comparatively little research has been done on pigmentation in sheep and it is only within the last few years that any really comprehensive study of the inheritance of colour and colour pattern has been made (Roberts78-84 Wassin 96). Roberts' series forms an important contribution to the Genetics of the Sheep but whilst explaining how colour is inherited, genetics cannot yet suggest its control. The question then arises - Is control necessary? Most sheep breeders might answer in the negative, but on consideration, the desirability and even the necessity for colour control, become obvious. Even in Britain, where the sheep is farmed for mutton firstly, and wool secondarily, colour is of very real significance. It is a common theory (and like many popular beliefs possibly has/

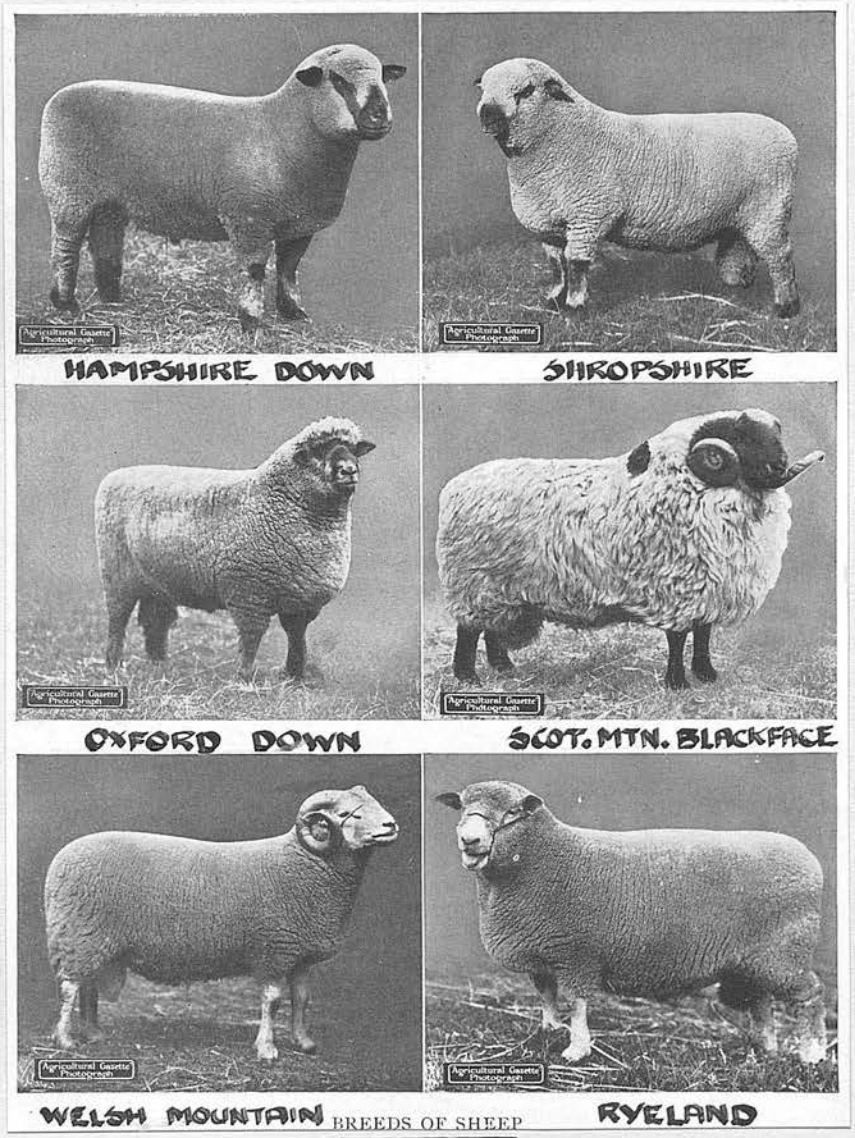


FIG. 7

from Blacks Veterinary Dictionary

has a scientific basis) that the quality of the mutton is influenced by the colour of the sheep, and a dark-faced sheep is on such grounds, always preferred to a white-faced one. In addition to this, in the fixation and definition of breeds a distinctive colour is a highly desirable character. This fact has, however, no universal application and in a highly specialized wool sheep such as the Merino, colour is the least welcome attribute since the use for which the wool is destined, to a large extent determines the importance of the presence of colour in the fleece of the sheep. Even in one of Britain's whitest sheep, the Cheviot, colour spots and scattered coloured fibres do occur. Why is it then that even the whitest sheep is not free from colour? It is simply that genetically and basically the sheep is coloured, and whiteness or any modification of colour is merely an imposed character. This persistence of a genetic colour factor is not easily explicable since in every other group of animals albinos or recessives occur with varying frequency and inevitably the thought arises that as suggested by Roberts (83) recessive white sheep must exist but have yet to be described.

III(a) i. Notes on Colour in Specific Breeds of Sheep.

In Britain, since the sheep is primarily farmed for/

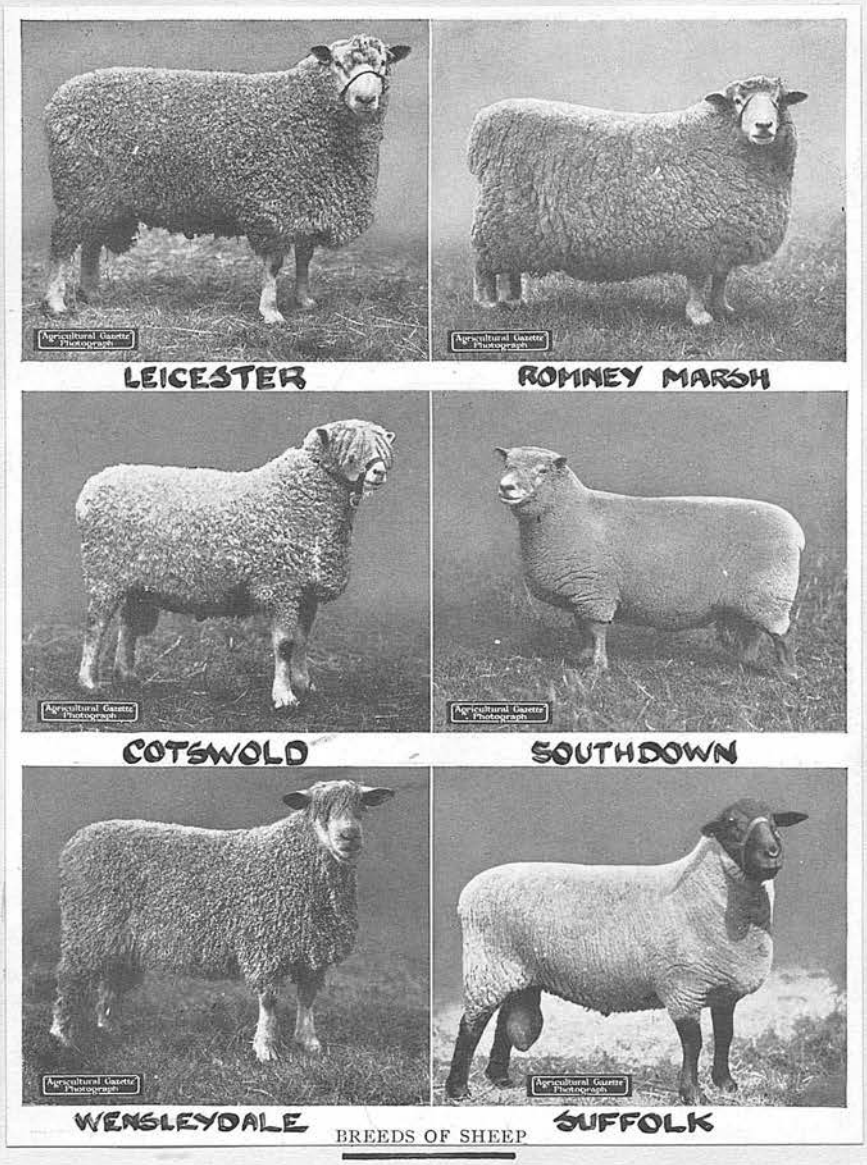


FIG. 8

from Black's Veterinary Dictionary

for its mutton producing qualities, wool production is only of secondary consideration. This being the case, standardisation of breeds and their improvement have been undertaken mostly from the point of view of the carcase and not its covering, but in spite of this fact the fleece is of very real commercial importance. In this respect it is required that every breed of sheep shall conform to certain standards in the particular character of its wool. Specifically, the British breeds of sheep may be grouped into Longwoolled, Mountain and Down breeds all more or less white woolled sheep but with a varying amount of colour on heads and legs. As previously stated, the pure white sheep or albino has yet to be recorded. In breed classification therefore, colour has a very definite significance.

Amongst the Longwoolled sheep great variation exists in the distribution of colour, for although they are on the whole white, a certain degree of pigmentation occurs in each. After the Lincoln Longwool, the Roscommon is probably the whitest. It is not unusual to find the lips and nostrils pigmented as in the English Leicester, Border Leicester and Romney Marsh, and associated with this there is often a bluish tinge on the face, and occasional black spots on otherwise white ears. This tendency towards ear spotting/

spotting is also sometimes exhibited in the South Devon. Colour occasionally appears on the legs as well as the face and in the Cotswold, grey marking used to be not uncommon although at the present day they are rare. In the Devon Longwool the face and legs are not unusually of a brown coloration.

The most interesting Longwoolled breed, in so far as colour is concerned, is undoubtedly the Wensleydale. This is a white breed characterised by a skin of a deep blue colour on the face, ears and legs. Originally the breed was completely white, the colour being introduced by a half-bred Leicester ram with a dark blue head and nearly black skin. The deep blue face colour is to-day a desirable feature of those Wensleydales which are used by the breeder of cross-bred lambs. Unfortunately the occurrence of black lambs in pedigreed Wensleydale flocks is not at all unusual and has been made the subject of several extensive studies (11-14) The condition is discussed later in this paper.

The Cheviot is the whitest of all Mountain breeds but even here coloured spots may be found on the face or inside the ears and in some flocks the occurrence of recessive black Cheviot lambs is not uncommon.

The Exmoor Horn has scattered black spots on an otherwise white skin. The White Welsh Mountain may be either wholly white or have tan-coloured face and legs/

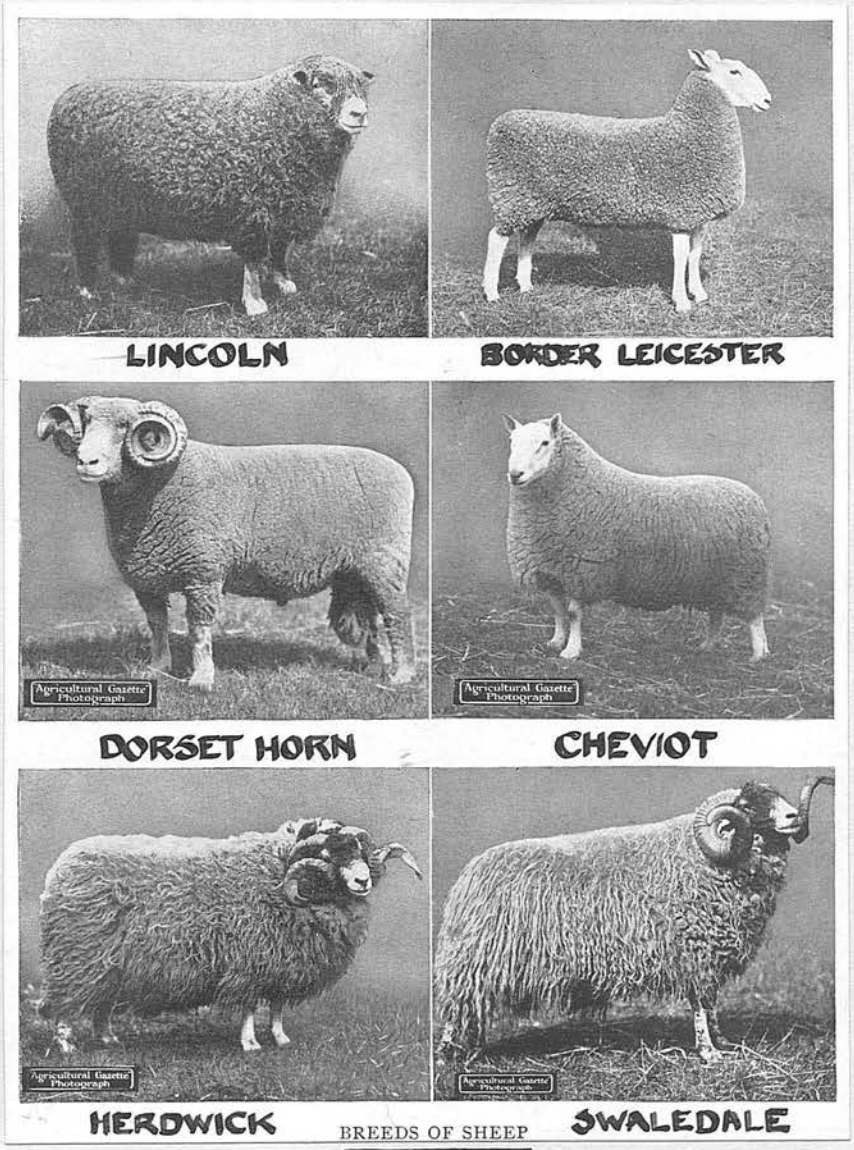


FIG. 9

from Black's Veterinary Dictionary

legs. It is usual for most other mountain breeds to have coloured faces and legs. The Scottish Mountain Blackface breed shows a wide range of variation in face colour, from an entirely black head, neck and legs, to black with very extensive white markings - an intermediate between the two is very common. Spots elsewhere on the body are undesirable but not infrequent. In the Lonk the white markings on the black face and legs are well defined, and the coloration in the Rough Fell is somewhat similar except that the muzzle of the latter is always white or grey. The face of the Swaledale may be either black or dark grey and the nose is often described as mealy, in this respect resembling certain of the Scots Blackfaced sheep. The Gritstone is characterised by a dark or mottled face and legs with patches of black wool not unusually occurring on the belly thigh and rump. Although the usual colour on these parts is black an undesirable brown sometimes replaces it.

Transitional colour changes which take place during the development of the sheep are of a high order of significance and the condition in the Herdwick is especially interesting. At birth the lamb has a blue black head and legs with black or grey wool on the body. The ears are white-tipped and the hoofs are fringed/

fringed with white hair. Whitening extends from these areas, till, at the end of the fourth year of life, the face and legs of the adult sheep are white or grey.

Each of the Down breeds shows some colour on the face and legs and since they produce finer wool than either the Longwoolled or Mountain sheep the occurrence of scattered coloured patches in the fleece of these is of relatively greater importance.

The Southdown in which face and legs are of an even mousey colour, is characterised by possessing a fine white wool. In the Shropshire the face and legs are black whilst in the Hampshire Down the colour may be dark brown or black. Both the Oxford Down and the Dorset Down are characterised by a dark face and legs and in the Suffolk the head and legs are intensely black and the body white. The Suffolk lamb, however, is born with a dark brown, black or speckled coat and it is only gradually that the characteristic coloration of the adult fleece is assumed.

Other breeds which resemble somewhat the Down, and which are not easily grouped in any of the three main breed classes, are the Dorset Horn, where the face and nose are white, the Ryeland, which is characterised by a dull white face and legs and the Kerryhill/

Kerryhill where the white face is often speckled with black or grey on the extremities.

The foregoing review does not include all the British breeds of sheep but the descriptions are sufficient to illustrate the part that colour plays in breed standardisation.

A few flocks of self-coloured sheep are still in existence, e.g., Shetland, Soay, Black Welsh, and in white-fleeced flocks it is not exceptional to find coloured lambs. The wool from such sheep is never dyed and has certain specialized uses.

In Australia and South Africa are to be found the finest wool-producing sheep in the world - Merinos. Characteristically the breed is white but black lambs occasionally crop up and scattered coloured fibres are not unknown, even in the finest fleeces. This occurrence of scattered coloured fibres which is a grave defect from the manufacturers' point of view finds a parallel in all British breeds of white fleeced sheep. The Suffolk may be quoted as a British breed in which coloured fibres are especially undesirable and lately, coloured fibres in the Scottish Mountain Blackface breed have come to be regarded with great disfavour. It is naturally the use to which the wool is to be put that determines the relative importance/

importance of the occurrence of these scattered coloured fibres.

IIIa. ii Economics.

The economic aspect of pigmentation in the fleece of the sheep must not be disregarded. Whether it be mutton quality or wool standards which are under consideration, the colour of the sheep must be taken into account. Breeders have very distinct preferences for certain types of sheep and many believe that there exists a very real correlation between colour and mutton quality. Other things being equal, a dark faced Scots Blackface is preferred to a white faced one in that the former is said to be hardier than the latter. On the other hand light faced ewes are preferred to those with a darker colour, since it is thought that light face-colour is indicative of a capacity for better milk production which in turn ensures a better chance of better lambs.

Where pedigree stud flocks are maintained the loss entailed in eliminating otherwise valuable lambs on account of the undesirable colour of the fleece must be great. The Suffolk breeder knows that it is inadvisable to retain badly spotted lambs since the adult fleece in such is never really clean; this is particularly true of those lambs which show brown spots. In cross-bred Suffolk x Half-bred flocks the "brockit-faced" lamb fattens quicker than those with a dun/

dun-coloured face and realises a better price on the market.

The appearance of black lambs, occasionally to the extent of 15 per cent, in Wensleydale flocks is undoubtedly a serious problem for the breeder, but it is hoped, not an insoluble one. It has been stated more than once, that the practice of breeding sheep with certain well defined colour characteristics and the necessity for maintaining these colour patterns as breed characters, is at the root of the difficulty.

Prof. A.F. Barker in his report on "Points for Selection by Breeders", 1930, stresses the possibility of selecting breeding sheep with too much black on the face and specially advises against those where the colour tends to creep down the neck. Moreover, where a potentially functioning pigmentary system is present it is almost impossible to restrict its activities to certain limited areas of the body, and this is directly responsible, not only for the production of coloured lambs, but also for the appearance of the scattered coloured fibre. Suffolk wool, on account of its excellent quality and fineness is extensively used in the manufacture of hosiery goods and the presence of coloured fibres greatly detracts from the value of the fleece.

The most serious aspect of this scattered coloured fibre/

fibre problem is undoubtedly concerned with Merino wool production. Merino wool enjoys a world-famous reputation as the finest white wool in existence and is, to the exclusion of all other wools, used in the manufacture of materials known as "natural creams". These "natural creams", in the manufacture of which only 70^{s} quality wool and over is used, undergo no dyeing process, so that the presence of even a few coloured fibres is startlingly obvious. Since neither peroxide baths nor sulphur stoving will bleach these coloured fibres, each one must be removed by hand, an expensive and tedious process. It is estimated that money lost through the necessity for this "burling and picking" process amounts to several thousand pounds per annum.

Scattered coloured fibres constituted such a serious defect in South African Merino wool, that it is only within the last ten years (during which time breeding practices have been tremendously improved) that manufacturers have been able to risk its use in the manufacture of "natural creams". South African breeders therefore, should continue to employ every means of eradicating colour from the Merino fleece.

Professor Barker in his report on "The Sheep and Wool of South Africa, Rhodesia and Kenya Colony 1930", deprecates the practice which is presently in vogue of crossing/

crossing blackhead Persians and Suffolks with the Merino, and, with other authorities, stresses the danger that exists of permanently impairing the whiteness of the merino fleece. He mentions the appearance of black lambs in pedigreed Merino stock imported from Australia and indicates the consequence of ill-considered breeding practice. The analysis of coloured fibres in the Suffolk which follows gives some little idea of the value of a knowledge of the fleece characters of a sheep, especially when such sheep are to be used in cross breeding.

IIIb. THE APPEARANCE AND BEHAVIOUR OF PIGMENT IN THE SUFFOLK BREED.

The colour changes exhibited by the Herdwick and Suffolk, during the transition of the birthcoat to that of the adult, are a direct expression of metabolic function. Their definite correlation with the age of the individual is very striking, and it was thought that an investigation of the character of the fleece during the period of colour change would throw further light on the pigmentary system of the sheep. Nichols (64) published a paper on "The Occurrence of Dark Fibres in the Suffolk Fleece with particular Reference to the Birthcoat of the Lamb" and for this reason it was decided to select the Suffolk, in preference to the Herdwick for further research.

The Suffolk originated from a cross between the Old Norfolk Horn and the Southdown, and for the last seventy years has been recognised as a standard breed of sheep.

In addition to its excellent mutton qualities, it possesses an extremely desirable fleece of fine white wool, which in common with that of other Down breeds approaches more nearly to that of the Merino than many other British breeds of sheep. The Suffolk is presently greatly in demand for export purposes and its/

its use for crossing with other breeds is being greatly extended. For example, this is especially the case in South Africa where experimental crossing with Merino and Native sheep, with a view to producing a "dual purpose" sheep, is being undertaken. The character of the cross-bred fleece is in these cases a matter of primary importance and it is essential that the breeder should understand the nature of the parent fleece, and in this connection the behaviour of the pigmentary system is of the greatest importance.

In the adult, the face and legs are uniformly black whilst the body is covered with fine white wool and a small quantity of fine white wool in the form of a tuft sometimes occurs on the head. The birthcoat of the lamb is however quite different; it may be of a uniform dun colour, speckled black and white, or pure black.

Five lambs, approximately eight weeks old, were selected from a flock of pedigreed Suffolks for observation. In a preliminary study a brief analysis of samples of wool from each parent was made. In each case a very small percentage of both black and white kemp was present; a few completely pigmented wool fibres occurred and there were several white wool fibres with coloured tips; in other respects the wool was/

was distinctly clean and very fine.

Eight samples were taken from each lamb at approximately monthly intervals during a period of a year, June 1929 to May 1930 and carefully analysed. The sampling areas were:-

1. Median dorsal neck.
2. " ventral throat.
3. Left Shoulder.
4. Left flank.
5. Left Rump.
6. Britch.
7. Belly.
8. Left thigh.

For all practical purposes it was found that three samples (1, 3, 6) from each would have been sufficient.

The practice followed was to make general observations on the sample, always from an area about two centimetres square, comprising about 3500 fibres and then to remove from it at randon a hundred fibres. The special interest in this study was found in the microscope examination and analysis of the fibre types. Nichols defined three such types, as follows:-

1. Kemp.
2. Wool Fibres with pigmented tips.
3. Finely medullated, coloured, coarser fibres. - Intermediates.

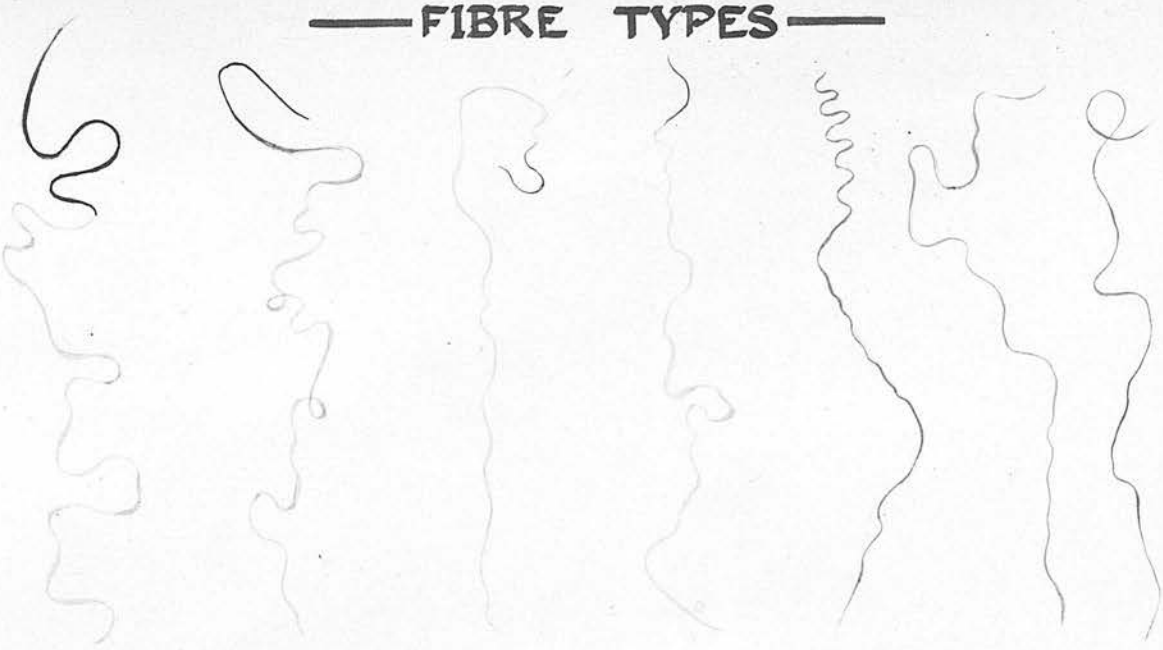
After the first two months and for the remainder of the year, all the kemp fibres in the samples were deliberately picked out together with every other obviously coloured fibre, and a number of completely white fibres. The gradual decrease in the proportion of/

of coloured fibres made such a selection desirable. For microscopic examination the fibres were washed in ether (the whole sample had previously been washed in warm benzene and then water) and then mounts were made in glyco-alcohol, euparal and cedarwood oil. Strong sulphuric acid, followed by gentle heating, was used for fibre disintegration work. As each set of samples was analysed the results were summarised and compared.

From the outset one lamb showed considerably less pigmentation than the others and a year later was possessed of easily the cleanest fleece. If this may be regarded as typical, the significance of an early analysis of the lamb's coat in predicting the colour character of the fleece of the adult is obvious. In March, 1930, the britch sample from this same lamb included a patch of coloured fibres but apparently such promiscuous spotting is inevitable in sheep which genetically carry colour factors. The shoulder itself is not a good colour indicator since that particular area clears very rapidly and even in sheep from commercial Suffolk flocks has only a very small percentage of coloured fibres.

Colour in the various fibre types.

The three types of fibres occur in both lamb and adult fleeces, although type 3, the finely medullated, coloured/

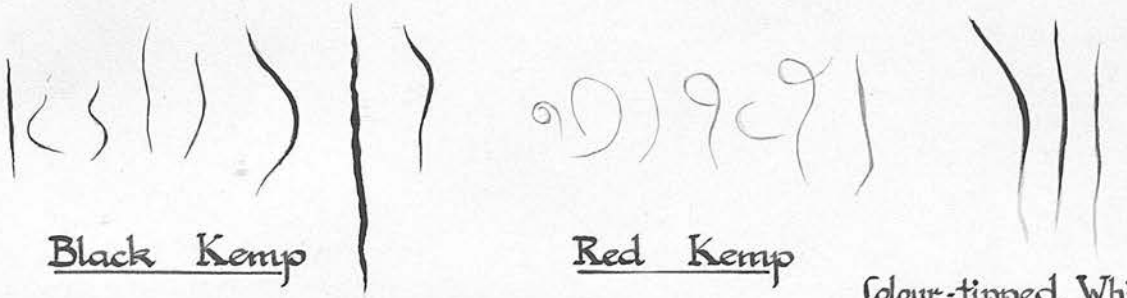


Colour-tipped Wool

Coloured Wool

Banded Wool

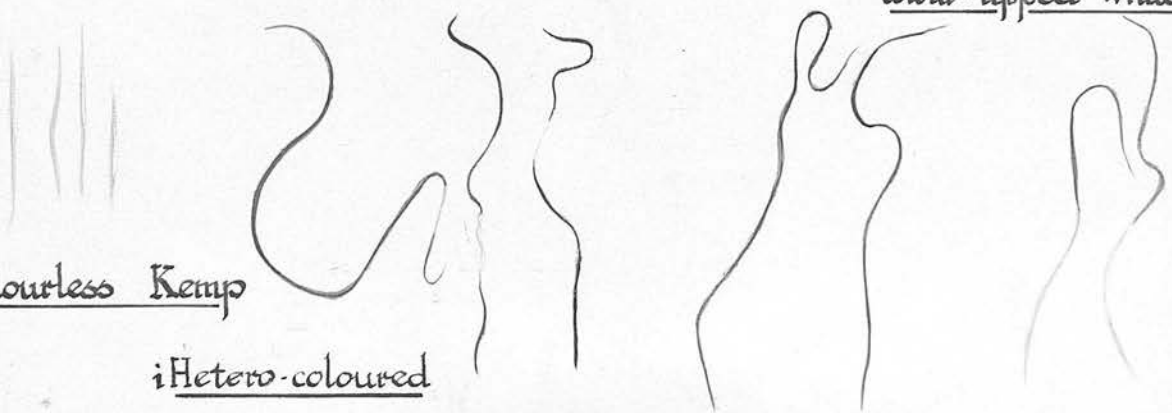
Bi-partite Wool



Black Kemp

Red Kemp

Colour-tipped White Kemp



Colourless Kemp

i Hetero-coloured

Intermediates: ii Banded iii Coloured iv Bi-partite

FIG 10

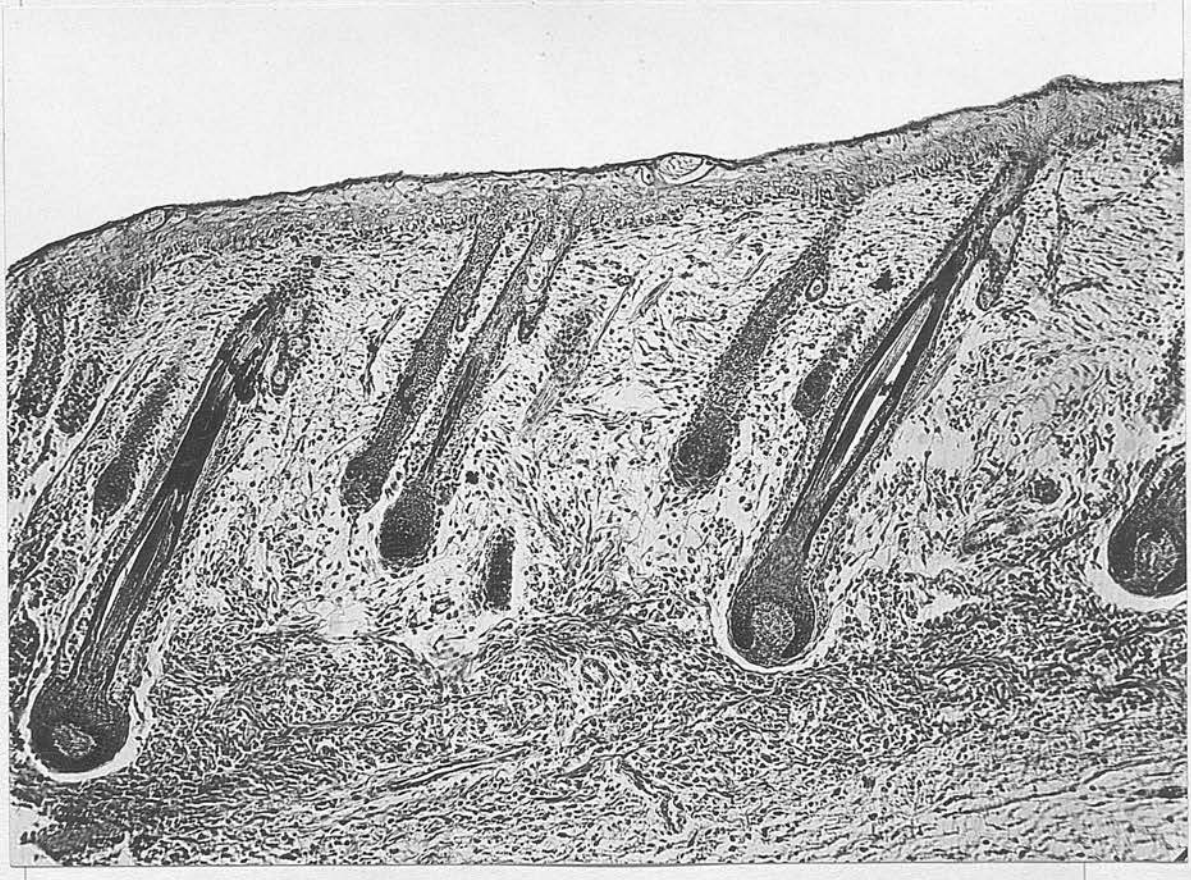


Figure 11. Microphotograph of skin section cut longitudinally through the fibre follicles; from shoulder of a 12 weeks' old Suffolk foetus. x circa 400.

coloured, and somewhat coarse fibres, is reduced to a minimum in the adult. In addition, completely coloured true wool fibres which are not present in the birthcoat are found in the adult fleece. Apart from the successive generations of coloured kemp fibres described by Nichols (64) which occur in the fleece as a whole, it was found that kemps showing only a small extent of pigmentation were specially prevalent in the axillary regions and to a lesser extent on the belly. It was on these regions also that the apparently white kemps occurred. Throughout the life of the Suffolk the ventral portion of the fleece is always darker than elsewhere and there is a greater preponderance of the pigmented finely medullated type of fibre on the belly.

Type I - Kemp.

The kemp series exhibited extreme variation both in form and colour (Fig. 10). They were found to be sometimes blacktipped or entirely white, black, brown, fawn or red. They might be stiff and straight and short, short and wavy, or irregularly crimped, or in some cases showed elbowing.

Type II - Wool.

The wool fibres in the lamb coat were all colour tipped, the coloured distal portion sometimes extending practically the whole length of the fibre. (Fig. 10).

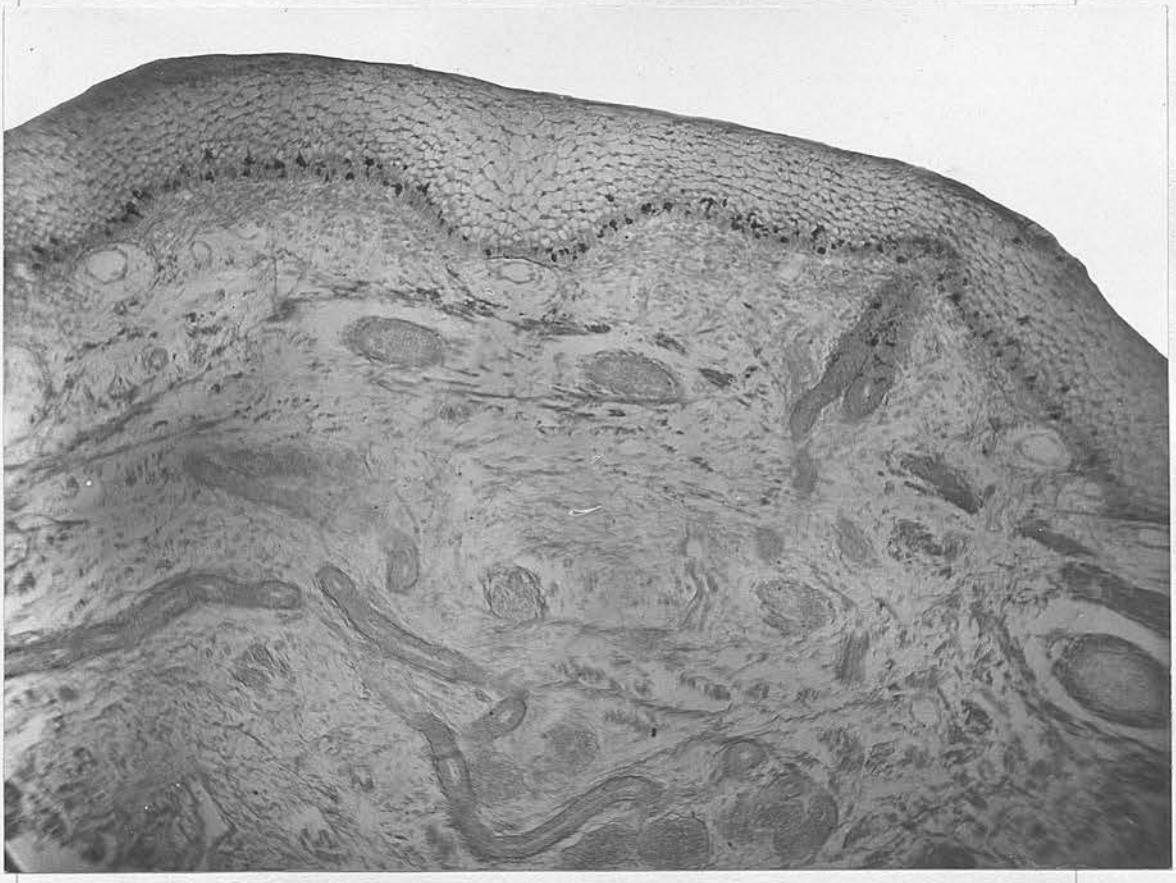


Figure 12. Microphotograph of foetal lamb's skin illustrating the stage of restriction of pigmentogenic cells to the Malpighian layer of the epidermis.

Often these coloured tips, which are easily broken off, were found lying free in the sample, but it was not difficult to distinguish them from coloured kemps.

Type III - Medullated Fibres.

These fibres exhibited extreme variation. (Fig. 10). In some instances they were long, coloured (black, red, etc.) hair-like, and often exceeded the length of the wool fibres. In others they were heterotypic in colour, being fawn distally and grey proximally, or black distally and white proximally or showed intermittent banding. Some were even heterotypic in form, strongly resembling, save in colour, the heterotype of the Merino (Duerden 16), being stiff and straight distally, and finely crimped and woolly, proximally. These fibres varied tremendously in diameter.

During the first few months there is a gradual decrease in the quantity of colour tipped wool and coloured fibres generally, and the character of the adult fleece becomes more and more defined by the increase in the fine white wool. Nichols' work indicates that the follicles of the intermediate coloured fibre type give rise to wool fibres. This is supported by the presence and appearance of colourtipped wool fibres in the fleece throughout the life/

life of the adult, the colour tipping being due to the fact that the cells producing such fibres are potentially pigment producing.

In the samples examined, every conceivable grade of colour was seen in the pigmented fibres although the kemp series were usually red, black or "colourless" (i.e. to the naked eye). There was extreme variation in the pigmentation of the wool and bipartites * and banded fibres were often found. The significance of the occurrence of colourtipped white kemp fibres is discussed later.

The minute details of deposition and disposition of pigment in the fibres were carefully studied and it was soon evident that no apparent genetic significance could be attached to these characters, since both the morphology of pigment granules and their arrangement in a fibre seem to be fortuitous. The part that structural characters of the fibre and quantitative differences in pigment may take in determining the ultimate colour of any particular fibre has already been referred to (See Pp. 9-11) and in the Suffolk the varying shades black, brown, red and fawn find in them a partial explanation. It is not yet known at what stage/

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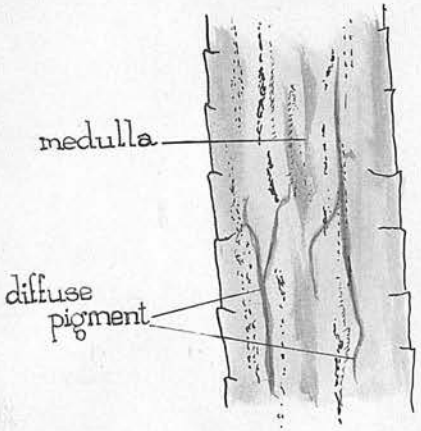
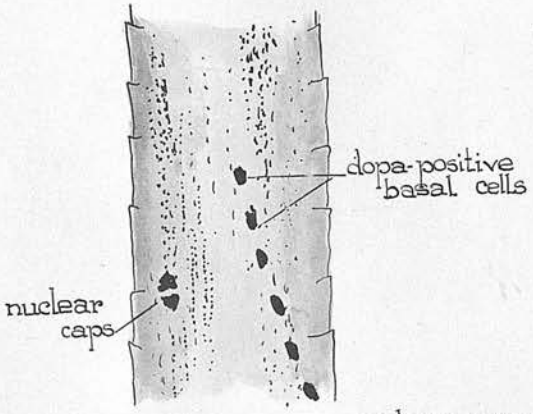
* Fibres approximately half-coloured and half-white, at the time of examination.

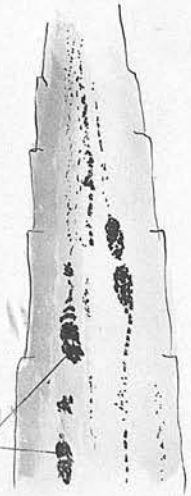
stage the differentiation of fibre cells into cuticle, cortex and medulla takes place and what it is that determines that either cortex or medulla or both shall contain pigment. In the Suffolk it would appear to be a matter of chance. In other animals, however, where self-colour is a genetic and fixed character, the inclusion and manner of deposition of pigment in the cortex only or the medulla only, or both, are within limits, predetermined, and it is this maintenance of pigment deposition and disposition which preserved breed or species coloration, so that the Aberdeen Angus, for example, is black, the Jersey is a reddish fawn, and, possibly most static of all, the Suffolk horse is chestnut. Variously coloured mice illustrate this point very clearly. Brown, black, grey and fawn mice owe their apparently different colours only to the variation in quantity and disposition of the pigment in the fibres.

The fortuitous manner in which pigment is included in the Suffolk fibre explains why any single fibre may exhibit profound colour differences along its length. It is significant that not in any wool fibres examined did pigment occur in the cuticle, although Hausman (32) suggests that occasionally, although very rarely, the cuticular scales of infrahominid mammals may be tinted as though stained

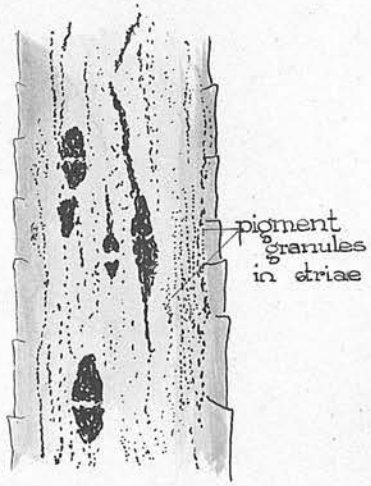
by/

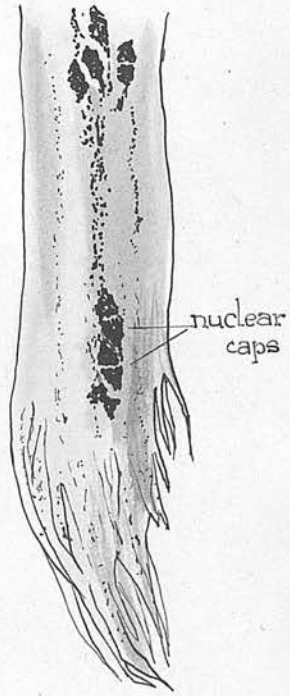




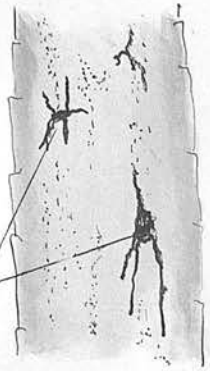


Tip of Fibre - [a]

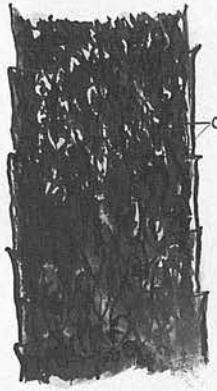




Brush end of [a] showing pigmentation



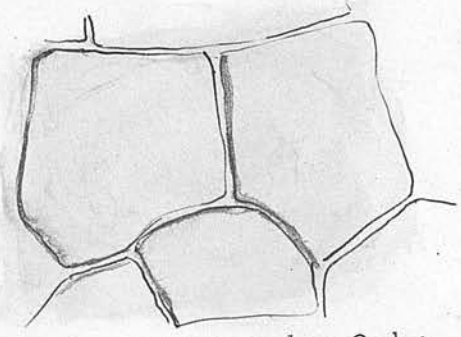
Lightly pigmented fibre



Heavily pigmented fibre

Single, pigmented Cortical Cell [x 1000]

Middle portion [a]



Colourless, flat Cuticular Scales [x 1000]

FIG 13 TYPES OF PIGMENTATION IN FIBRES FROM SUFFOLK WOOL

by diffuse pigment, and he records a sparse occurrence of pigment granules in the transparent keratin of the cuticle of Chinese and Japanese human hairs. It is obvious that the structure of the cuticle in wool fibres, at least, does not permit of pigmentation. During the study of the histology of the developing Suffolk fibre which was carried out in connection with this work particular attention was paid to this point. Papillal cells which later gave rise to cuticular scales were observed to contain pigment. Extended observations showed, however, that as these cells moved upwards in the follicle the pigment either migrated into the cortex or was pressed out into the intercellular spaces or else migrated to the dermis, where it was phagocytosed. The thin leaf-like structure of a cuticular scale conclusively shows that pigment inclusions are a physical impossibility. (Fig. 13).

Pigment granules may be so small as to be unmeasurable, so fine as to be invisible, save as a diffuse stain, or so large as to obscure cell structure. They may occur in such quantity as to deform the natural profile of a fibre, or be so few in number as to leave the fibre colourless. When pigment appears to assume a definite regular shape the cause may/

may often be attributed to the structural restrictions of the cells of the fibre, and in the Suffolk where heavily pigmented fibres appear to contain spindle shaped pigment masses (Nichols) in reality the spindle form is defined by the spindle-shaped cortical cells. (Fig. 13). On the other hand, many fibres do appear to exhibit a definite arrangement of pigment granules and a linear grouping is often found. Such an arrangement is however only mechanical and illustrative of the compression and subsequent elongation of the fibre cells as they are pushed upwards from the apex of the papilla, preceding keratinization.

When diffuse pigment is produced in small quantities it assumes a characteristic streak-like appearance, due to the fact that during shrinkage of the fibre cells just at the time when keratinization occurs, the diffuse nature of the pigment allows it to seep through and flow into the cellular interspaces. (Fig. 13). In this study hundreds of fibres were disintegrated in hot concentrated sulphuric acid, and the behaviour of the pigment granules studied. Diffuse pigment under these conditions is readily soluble, whilst granular pigment remains insoluble

The presence of pigment aggregations in the fibre in the form of apposed bell-shaped masses was at first somewhat puzzling. (Fig. 13). They occur in practically/

practically every pigmented fibre, but are best seen in lightly pigmented fibres where their outline is not obscured by quantities of pigment inclusions. Later investigations of the histology of the skin and its developing follicles explained their appearance. These bell-shaped masses are formed by the aggregation of pigment granules over the nucleus of the apical cells of the papillae and their maintenance in such a position suggests that they are protective in function.

During development of the fibre many pigmentogenic cells pass up from the bulb unchanged, and keratinization, whilst destroying the protoplasmic contents of the cells does not affect the pigment inclusions, so that the original cell outline is preserved. It is not unusual to find series of these undifferentiated pigment cells lying along the length of a fibre, exactly similar in appearance to the basal dopa-positive cells of the epidermis. (Fig. 13).

Observations of dendritic pigment cells <u>in vitro</u> demonstrates quite conclusively the extraordinary activity and behaviour of pigment in the living cell and offers sufficient explanation for the extreme variation in deposition and disposition which is seen in the keratinized wool fibre.

The definition and assumption of the adult fleece depends on a restriction of the activities of the pigmentary/

pigmentary system. The genetic constitution of the Suffolk ensures that in the head and legs the inherited potentiality for pigment production shall always find expression, but that elsewhere the pigmentary system shall be more or less quiescent, responding only to definite additional stimuli. The phenomenon of self-colour in the young followed by an entirely different appearance in the adult is by no means restricted to sheep and is a not uncommon occurrence throughout the animal kingdom. It may be seen in birds, e.g. Gannet; rabbits, e.g. Argenté; Llamas, etc. The exhibition of a colour change during the first period of independent existence is naturally controlled by the genetic stability or versatility of the pigmentary system, and it may be assumed that where a retrogressive colour change does take place the pigmentary system fails to function because the chemical environment is unsuitable. That this environment could be controlled is probable. The colour change is correlated with general body development and it is reasonable to postulate that just as the maintenance of an axolotl in a certain environment prevents its assumption of adult characters, so would the retardation of development in the Suffolk, prolong the retention of a wholly pigmented fleece.

A/

A lack of comprehensive data makes it impossible to define precisely the nature of the interference which modifies the functioning of the pigmentary system, but whatever the cause, the immediate inhibitor is probably the inertia of the nucleus in so far as the production of the oxidising enzyme is concerned. Support for this theory is found in the fact that since scattered coloured fibres develop sporadically in the fleece the production of dioxyphenylalanine from tyrosin and the circulation of this substrate in the blood must be a constant occurrence. The appearance of colour tipped white wool fibres indicates that the intensive cellular activity which accompanies the initiation of fibre growth is correlated with pigment production. The variation in the length of the coloured tip however is more difficult to explain, but it must bear some relation to the strength of the original stimulus. Although we may accept the fact that dopa is always circulating in the blood a variation in quantity must be considered, and when only small amounts are present it is possible that not every cell nor every follicle receives its quota. This offers a further explanation for the occurrence of scattered pigment granules in practically colourless fibres, and for pigment being found along one side of

a/

a fibre only, in caps, in dense masses, etc. Since the characteristic metabolism of the body determines the quantity of protein which is available for melanin synthesis, it is possible that in those animals where adult and birth coat coloration differs, with development, the available melanin precursor is diminished. This indicates an interesting line for investigation.

All sheep even within a breed show great variation in the time at which the pigmentary system begins to function, but generally speaking pigment deposition occurs about the end of the first month of intra-uterine life. In the Suffolk it has been observed before the end of the fourth week in minute quantities round the lips, and as a rule the head and hoofs show pigmentation before the body generally.

It cannot be said that the Suffolk has ever been shown to exhibit the Schultz reaction and the presence of colour-tipped white wool fibres is in no way connected with the Schultz reaction. The regeneration of coloured or colour-tipped fibres in place of white ones after injury, after maggot attack involving shedding of the fleece, or shaving, is due to the increased cellular activity of the cutaneous system inducing a response on the part of the pigmentary system.

The Suffolk, in point of fact exhibits precisely the/

the reverse conditions of those that obtain in the Himalayan rabbit and in it, temperature cannot be considered even one of the many controlling factors in the activities of the pigmentary system. Nichols' postulation of "thresholds of irritation (minimal levels of metabolism for effective action, which can be affected by temperature)" is therefore not acceptable.

The browning of the hairy covering of the face which is sometimes seen in the Suffolk ewe after lambing is due to debility and therefore decreased metabolic activity and is somewhat comparable with the decoloration which accompanies age.

The microscopic survey of the fibres of the Suffolk has demonstrated within one breed, a variety of activity on the part of the pigmentary system, such as is almost inconceivable. It has proved of great value in estimating the nature of other colour phenomena, and has often prevented rash assumptions and inaccurate interpretations.

The description of the phenomenon of "Colour Banding in the Fleece" is one case in point, and since the completion of the Suffolk work, it has been possible to suggest an explanation of the phenomenon which before seemed incapable of interpretation. It does not appear to be extravagant to state that given the/

the suitable environment any coloured sheep would produce a banded fleece and remembering the occurrence of tipped and banded fibres in the Suffolk it would appear to be only a matter of degree; every variety of wool is possibly a banded wool, only the banding is restricted and therefore obscured.

Chart 3. To illustrate the distribution of coloured fibre types in Suffolk lamb I. The red crosses refer to the occurrence in the respective areas of the fibre types which they are opposite.

Column R records the occurrence of red coloration; the red figures refer to areas. For details of areas see page 90.

—LAMB I—

AREAS		MAY JUNE 1	2	3	4	5	6	7	8	R	JULY AUGUST 1	2	3	4	5	6	7	8	R
Wool:	Tipped	×		×	×		×			8	×	×		×		×	×		1
	Coloured																		2
	Banded																		4
																			5
Kemp:	Black	×		×	×			×	×		×	×	×		×		×	×	6
	Red		×																
	Colourless													×			×		
Black-tipped white																			
Intermediates:																			
	Coloured	×			×		×		×							×			
Hetero-coloured				×			×				×				×				
	Banded	×				×			×										
Bi-partites																			

AREAS		SEPTEMBER OCTOBER 1	2	3	4	5	6	7	8	R	NOVEMBER DECEMBER 1	2	3	4	5	6	7	8	R
Wool:	Tipped	×	×	×	×	×	×	×		1	×	×	×			×	×	×	1
	Coloured									8	×								2
	Banded																		3
																			5
Kemp:	Black	×	×		×	×	×		×		×	×	×	×			×	×	6
	Red														×				8
	Colourless							×											
Black-tipped white								×											
Intermediates																			
	Coloured								×			×						×	
Hetero-coloured																		×	
	Banded																		
Bi-partites															×				

AREAS		JANUARY FEBRUARY 1	2	3	4	5	6	7	8	R	MARCH APRIL 1	2	3	4	5	6	7	8	R
Wool:	Tipped		×	×	×	×	×	×	×	1	×	×	×	×	×	×	×	×	6
	Coloured									3									
	Banded									8									
Kemp:	Black	×	×	×		×	×	×	×		×	×			×	×		×	
	Red	×		×					×										
	Colourless												×						
Black-tipped white													×						
Intermediates																			
	Coloured					×													
Hetero-coloured									×										
	Banded	×																	
Bi-partites											×				×				

CHART III

Chart 4. To illustrate the distribution of coloured fibre types in Suffolk lamb II. (For description see Chart 3, page 108).

LAMB II

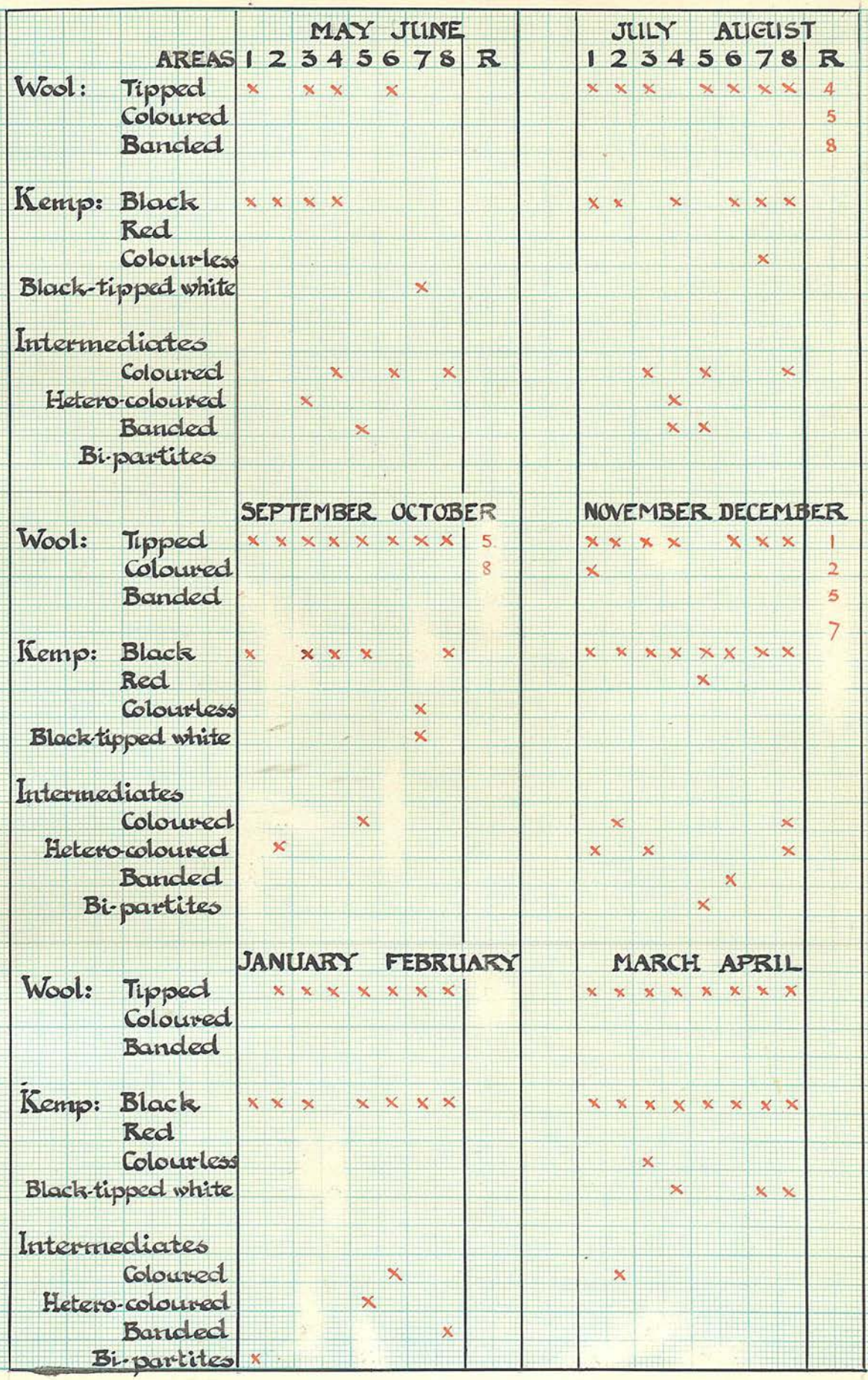


CHART 4

Chart 5. To illustrate the distribution of coloured fibre types in Suffolk lamb III. (For description see Chart 3, page 108)

—LAMB III—

MAY JUNE

AREAS	1	2	3	4	5	6	7	8	R
Wool: Tipped	×	×							1
Coloured									
Banded									
Kemp: Black	×	×	×	×	×				
Red									
Colourless									
Black-tipped white									
Intermediates:									
Coloured	×			×		×			
Hetero-coloured			×			×			
Banded				×					
Bi-partites									

JULY AUGUST

AREAS	1	2	3	4	5	6	7	8	R
Wool: Tipped	×	×	×	×	×		×	×	5
Coloured									
Banded									
Kemp: Black	×	×			×		×		
Red									
Colourless									
Black-tipped white									
Intermediates:									
Coloured	×		×		×		×	×	
Hetero-coloured						×		×	
Banded									
Bi-partites									

SEPTEMBER OCTOBER

AREAS	1	2	3	4	5	6	7	8	R
Wool: Tipped	×		×	×	×	×	×	×	5
Coloured									6
Banded									8
Kemp: Black	×	×		×				×	
Red									
Colourless			×						
Black-tipped white									
Intermediates									
Coloured						×			
Hetero-coloured		×							
Banded									
Bi-partites									

NOVEMBER DECEMBER

AREAS	1	2	3	4	5	6	7	8	R
Wool: Tipped	×	×	×	×	×	×	×		1
Coloured									5
Banded									6
									7
Kemp: Black	×				×	×	×	×	8
Red	×								
Colourless									
Black-tipped white									
Intermediates									
Coloured	×				×	×		×	
Hetero-coloured									
Banded							×		
Bi-partites									

JANUARY FEBRUARY

AREAS	1	2	3	4	5	6	7	8	R
Wool: Tipped	×	×	×	×	×		×	×	5
Coloured									7
Banded									
Kemp: Black	×	×							
Red				×					
Colourless							×		
Black-tipped white									
Intermediates									
Coloured									
Hetero-coloured									
Banded									
Bi-partites									

MARCH APRIL

AREAS	1	2	3	4	5	6	7	8	R
Wool: Tipped	×	×	×	×	×		×	×	4
Coloured									6
Banded									
Kemp: Black				×	×	×			
Red									
Colourless									
Black-tipped white							×		
Intermediates									
Coloured									
Hetero-coloured								×	
Banded									
Bi-partites									

CHART 5

Chart 6. To illustrate the distribution of of coloured fibre types in Suffolk lamb IV. (For description see Chart 3, page 108)

—LAMB IV—

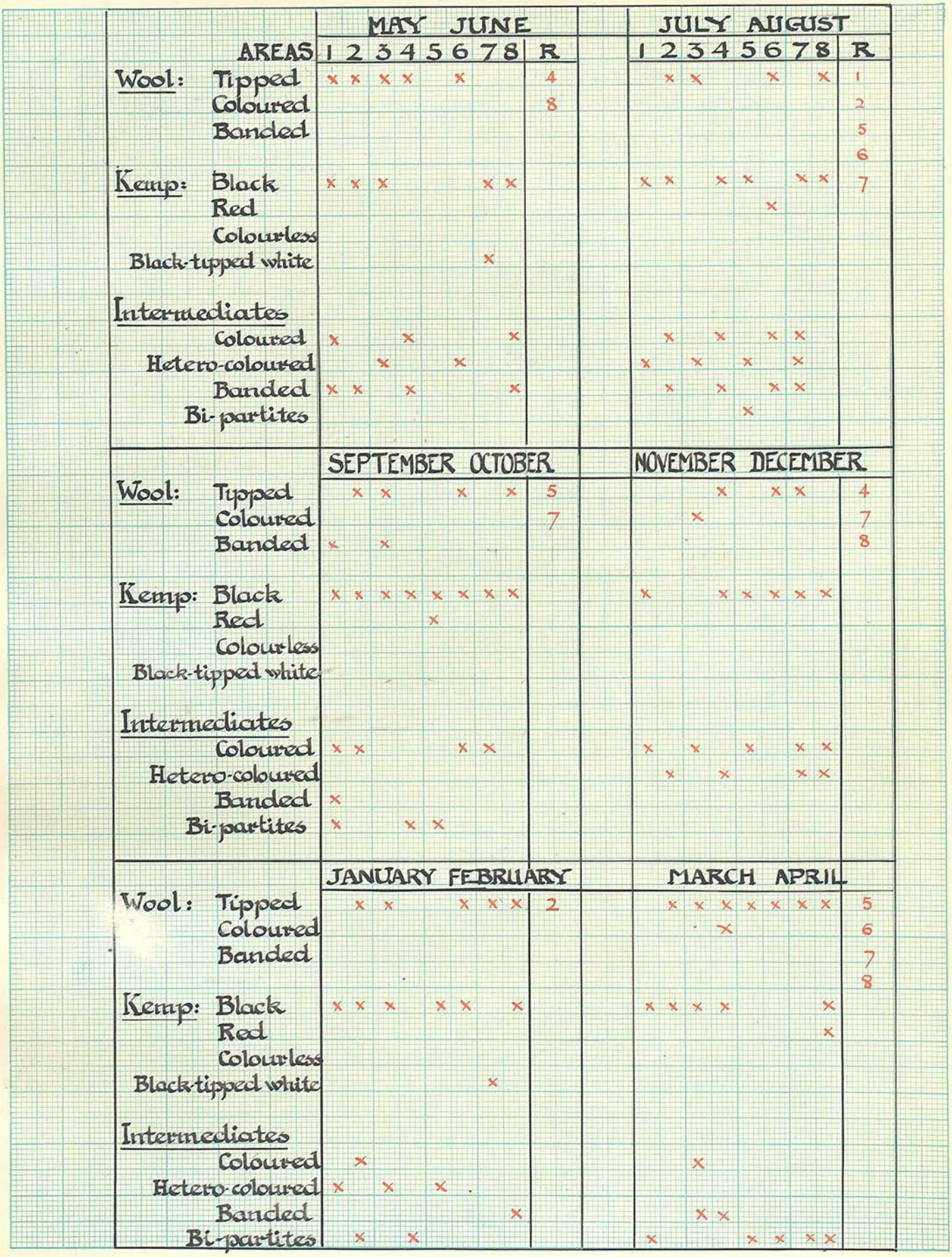


CHART 6

Chart 7. To illustrate the distribution of coloured fibre types in Suffolk lamb V. (For description see Chart 3, page 108)

—LAMB V—

		MAY JUNE									JULY AUGUST								
	AREAS	1	2	3	4	5	6	7	8	R	1	2	3	4	5	6	7	8	R
Wool:	Tipped	×		×			×			6				×			×		1
	Coloured									8	×								5
	Banded																		
Kemp:	Black		×																
	Red																		
	Colourless																		
Black-tipped white																			
Intermediates:																			
	Coloured								×		×							×	×
Hetero-coloured				×		×	×				×	×			×	×			
	Banded	×			×	×	×	×	×			×		×			×		×
Bi-partites																			

		SEPTEMBER OCTOBER									NOVEMBER DECEMBER								
		1	2	3	4	5	6	7	8	R	1	2	3	4	5	6	7	8	R
Wool:	Tipped	×								4			×	×			×		5
	Coloured	×		×	×					6									6
	Banded								×	7									7
																			8
Kemp:	Black							×			×	×			×		×	×	
	Red																		
	Colourless																		
Black-tipped white																			
Intermediates																			
	Coloured		×			×	×	×	×		×		×		×	×			
Hetero-coloured			×									×	×	×				×	
	Banded			×														×	×
Bi-partites							×												

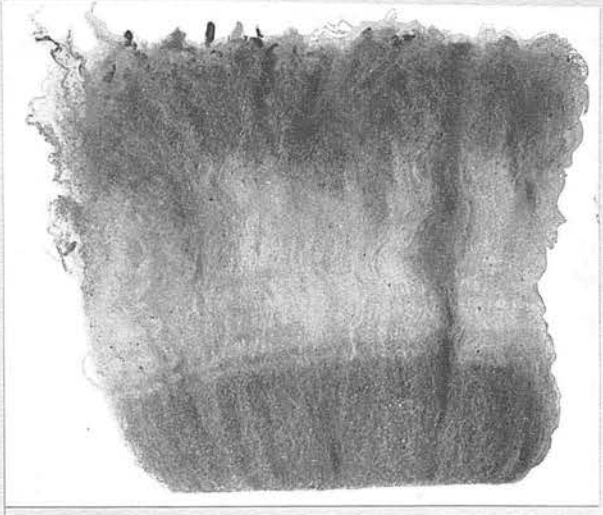
		JANUARY FEBRUARY									MARCH APRIL								
		1	2	3	4	5	6	7	8	R	1	2	3	4	5	6	7	8	R
Wool:	Tipped	×	×	×	×					5	×		×				×	×	4
	Coloured	×								6						×			6
	Banded									8									7
Kemp:	Black	×				×					×	×							
	Red																×		
	Colourless																		
Black-tipped white																	×		
Intermediates																			
	Coloured			×	×	×	×	×	×										
Hetero-coloured																			
	Banded	×						×								×			
Bi-partites		×	×	×	×	×	×				×	×	×	×	×		×	×	

CHART 7

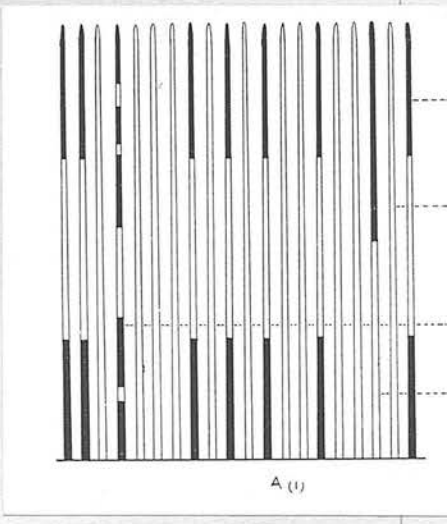
IIIc. COLOUR BANDING IN THE FLEECE OF THE SHEEP.

A banded wool is one in which there is an alternation between lighter and darker colours, an alternation which occurs more or less simultaneously over the whole fleece, and which results in the wool being traversed by well defined pigmented and non-pigmented bands. It is significant that in all the cases examined the outermost band formed by the tips of the wool was invariably coloured, and that, therefore, a sheep which produces a banded fleece always starts life as a coloured lamb. The simplest form of a banded fleece is produced when the coloured wool is traversed by a single white band, but in some fleeces there is a series of alternations and many bands are formed. There does not seem to be any limit to the number of bands which may be produced during the growth of the fleece, nor is the width of the bands the same, although the width of any particular band is more or less constant all over the body. In some cases the transition between band and band is clear-cut and well defined, but in others the outlines are fuzzy and indistinct. This is due to the fact that the individual fibres are not identical in their banding.

For the purpose of this study, five samples were used; four merinos (A, B, C, and D), and one coloured Hausa/

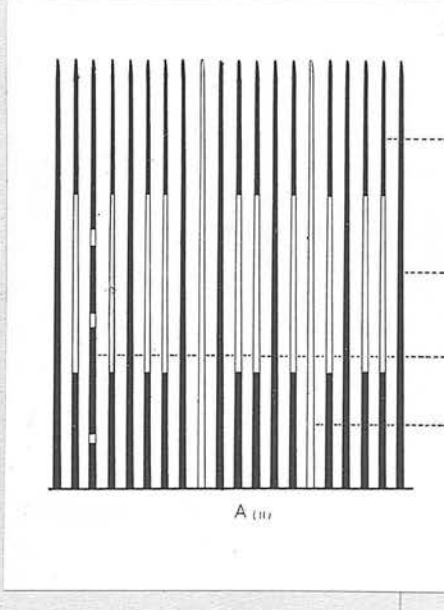


A(i) Shoulder



Fibre Analysis

A(ii) Belly



Fibre Analysis

Figure 14.

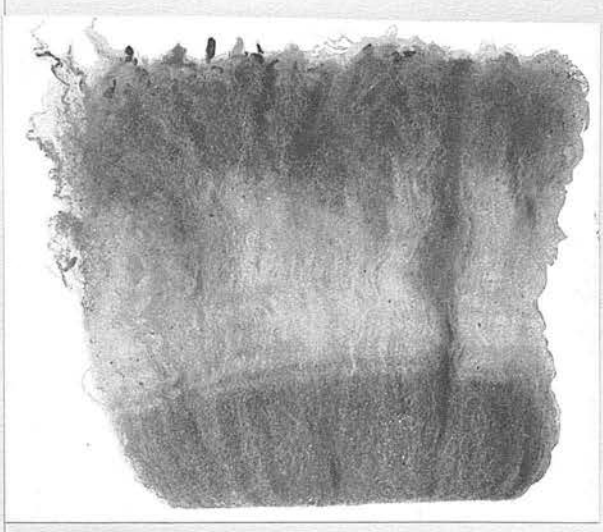
South Australian Merino Wool

Figures 14 - 16. Samples of banded wools. These show various types of banding encountered up to date. Alongside each is a schematic representation of the distribution of colour in the component fibres.

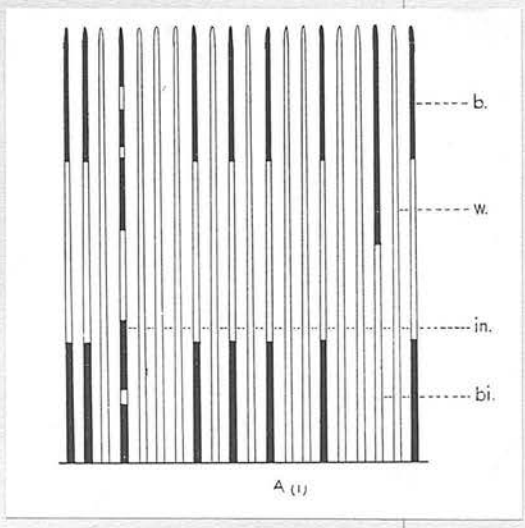
Letters refer as follows -

b.... ..banded fibres
w.....white fibres
in.....intermittently coloured fibres
bi.....bipartite fibres
c.....Wholly coloured fibres

Pages 120, 122 and 124.

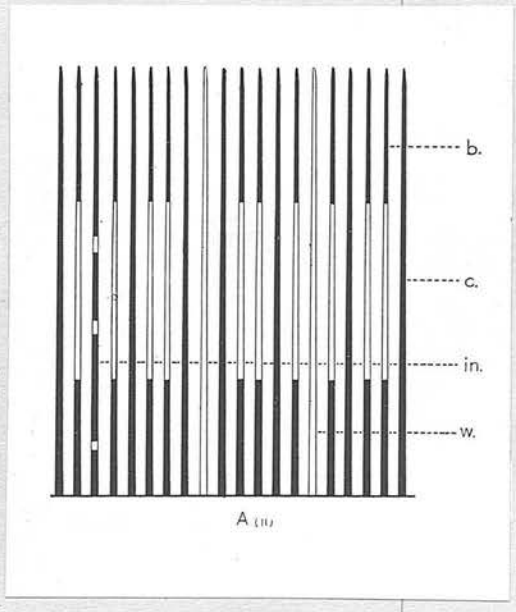


A(i) Shoulder



Fibre Analysis

A(ii) Belly



Fibre Analysis

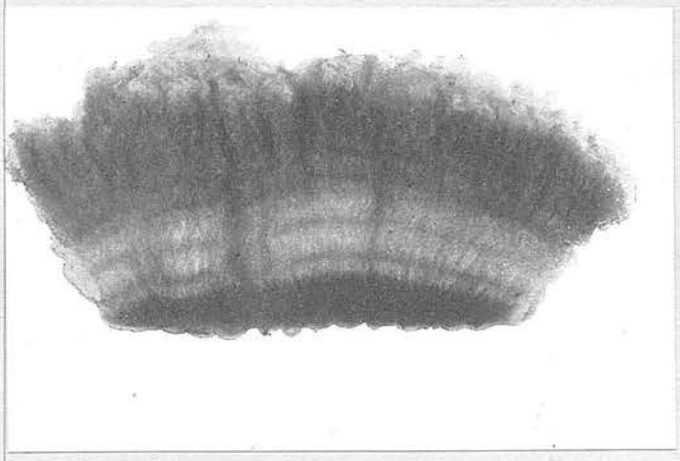
Figure 14.

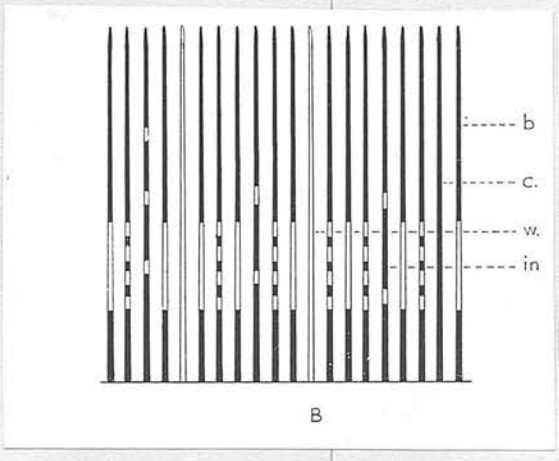
South Australian Merino Wool

Hausa x Shetland cross (E). In each case they were from fleeces which had never before been clipped, so that the tips of the fibres were still intact. A macroscopic and microscopic analysis was carried out, and after separation of the fibres it was possible to classify four fibre types which occurred in every sample, whilst in the case of two samples there was a small proportion of an additional fifth type.

Fibre Types.

(a) Banded Fibres. The banded fibre is typical for its particular sample, and, except in one instance, constitutes the bulk of the fibres. It is always coloured distally and the alternation of the coloured and colourless bars is the same as that of the sample as a whole. Since, however, no two fibres are identical, there are individual variations in the width of the bands. In C, where the banding is unusually well defined, the extreme limits of variability of the middle white band lie between 0.2 cm. and 1.6 cms., although more than 80% of the fibres lie between 1.0 cm. and 1.6 cms. Gross irregularity of contour characterises the great majority of banded fibres, but it is impossible to attach any importance to the thickening of a pigmented portion when, further along the length of the same fibre, a coloured portion occurs which is thinner than any other part of the fibre/

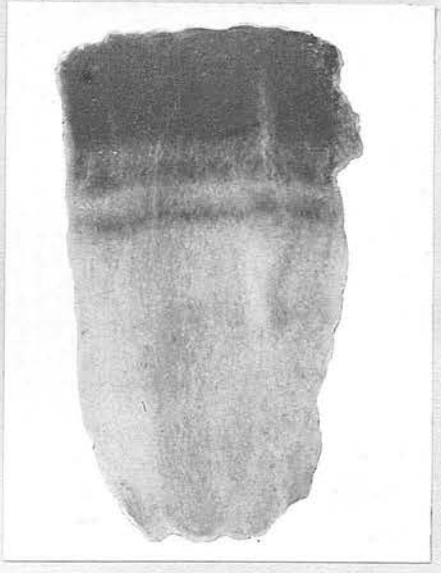


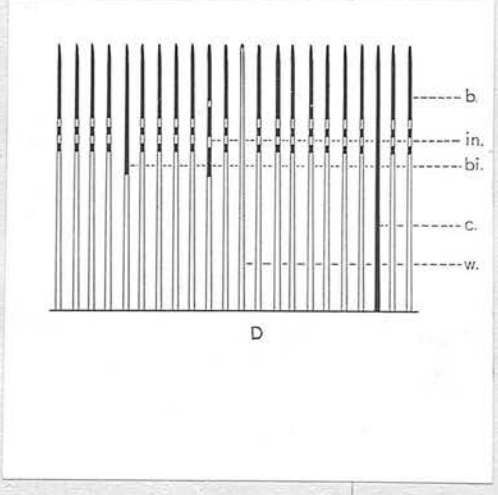


B

Fibre Analysis

Western Australian Merino Wool





Fibre Analysis

D

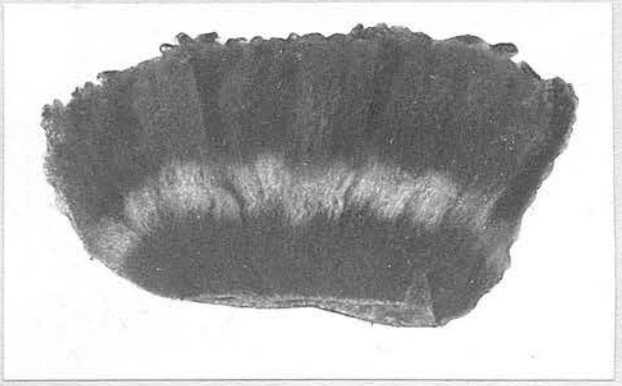
Figure 15.

South African Merino Wool

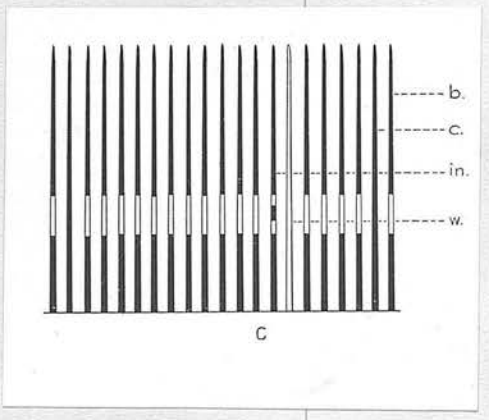
fibre, coloured or colourless.

The colour of the bands in any one fibre need not necessarily be the same, and there is often an appreciable difference between that of the distal band and the proximal, as in A and B, where many of the fibres show much diffuse pigment and less granular pigment distally, and a greater quantity of granular pigment proximally. When much diffuse pigment is present, the resulting colour is always lighter and brighter. In B it is necessary to define two types of banded fibre - (a) with proximal and distal coloured bands and clear median portion, and (b) complete with distal and proximal coloured bands and with three additional coloured bands running through the median colourless one. In 100 fibres, of which 72 were banded, 40 were of the (a) group, and 32 of the (b) group. Observation proves that an even smaller percentage of the (b) group fibres would have been sufficient to make the extra median bands apparent in the fleece.

It is not the proportion of banded fibres which defines the ultimate character of the fleece, for, whilst a few such fibres are sufficient to give a banded appearance to an otherwise white wool as in A (i), the effect of a large number of banded fibres is almost annulled by the presence of a few all-coloured fibres/

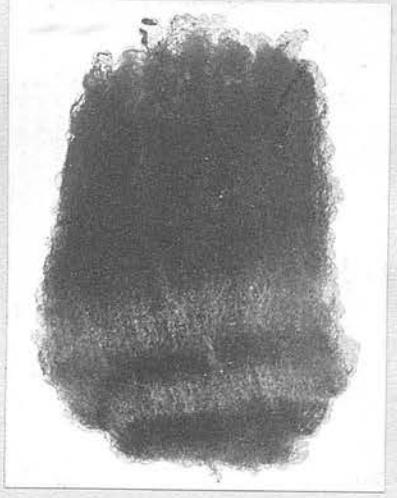


C.

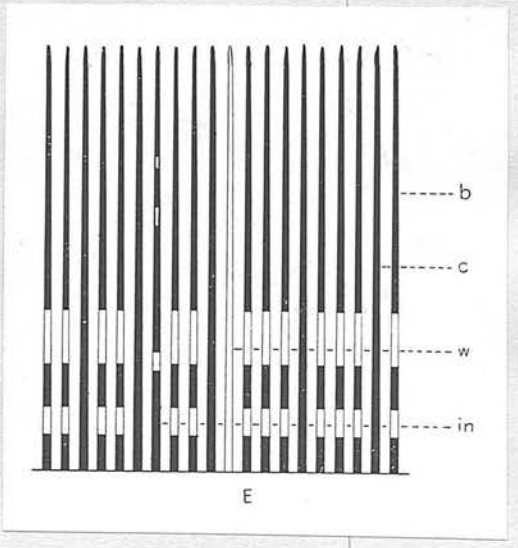


Fibre Analysis

South Australian Merino Wool



E



Fibre Analysis

Figure 16.

Hausa x Shetland Wool

fibres as in A (ii), and in some cases these are sufficient practically to obliterate the appearance of banding. In B, C, and D, where the banding is very distinct and colour contrasts are great, there are few completely colourless or completely coloured fibres, but in E, where the lighter bands are grey as opposed to the darker ones which are practically black, the dullness of the lighter bands is accounted for by the presence of many coloured fibres which detract from the white effect produced by the banded fibres. It is possible that many apparently self-coloured wools are really banded, and that the banding is masked by the aggregation of the coloured fibres. This, of course, could only be determined after an analysis had been completed.

Although, to the naked eye, the transition between the bands of the fibre is distinctly abrupt, microscopically there is a definite transition zone, highly variable in extent. In some cases there is a quick transition between pigmented and non-pigmented portions within a space of about 200μ, whilst in others the transition is so gradual as to be almost imperceptible, and the transition zone exceeds 1,000μ In many fibres the lighter bands are not really colourless, but contain a diminished quantity of granular/

granular pigment or in some cases merely diffuse pigment. When the coloured bands of a banded fibre are lighter than usual, it is sometimes found that there is a tendency for the pigment granules to lie along one side of the fibre only. This condition is specially prevalent in A and E.

(b) White Fibres. These were found to consist of two types, the one completely lacking pigment, and the other containing it in such small quantity as not appreciably to colour the fibre. In the pigmented white fibres, the pigment always takes the form of minute scattered granules, and the diffuse type is never seen. It is significant that a relatively large proportion of white fibres, such as occur in A (i), is not sufficient to obliterate banding, although it considerably modifies the colour of the distal and proximal bands, making them much lighter.

(c) Coloured Fibres. Under this heading are grouped all those fibres which are completely pigmented from tip to base. They vary in colour from lightest to darkest brown, and in E practically all the coloured fibres are nearly black. A few coloured fibres were found which gradually faded from dark brown at the tip to lighter fawn proximally. In both A (ii) and E, the proportion of coloured fibres to the rest of/

of the sample is relatively greater than that elsewhere, and the colour of the samples themselves is proportionately darker. In A (ii) these coloured fibres practically obliterate banding, whilst in E they darken the lighter bands to a dull grey colour.

There is considerable variation in the thickness of coloured fibres, the coarsest of them being thicker than the thickest fibre of any other type. When pigment deposition is unusually heavy, even a casual examination reveals irregular differences in thickness along the length of the fibre, and often the darker the fibre the greater its thickness. In D the all-coloured fibres are very much thicker distally, where pigment production is more or less uniform for the whole fleece, than proximally, where a distinct refinement is apparent.

(d) Intermittent Fibres. An intermittent fibre is one which is banded but irregularly so, and the alternation and number of the coloured and colourless bars differs to a greater or lesser extent from that of the banded fibres.

In C, the proximal and distal bands of the intermittent fibres are coincident with those of the banded fibres, but in addition, the median colourless bar is interrupted. This, however, is a somewhat extreme/

extreme case, and such a close correlation was not observed anywhere else.

Many of the intermittent fibres are hair-like in appearance and there is the greatest diversity in the colour of the pigmented parts. The percentage of these fibres is small, even in B where the largest number occurs.

(e) Bipartite Fibres. These are limited in their distribution and occur only in A and E. They exhibit the simplest of all types of banding, and there is but one colour alternation. Such fibres are pigmented distally and colourless proximally (a), or vice versa (b), and it is as usual to find the one type as the other. The line of demarcation between the bands is frequently found to lie about half-way along the fibre but this is not a constant characteristic.

It is apparent that notwithstanding the occurrence of many forms of banded wool, the phenomenon is in all cases essentially similar. Although all the fibres do not undergo a change at the same time, the majority do so with, however, considerable individual variation. This affects the delimitation of the bands of the fleece and when their outline is fuzzy and indistinct, it is directly due to this variable banding of the individual fibres.

The mechanism underlying the banding process is not/

Table Showing Analysis into Fibre Types

Sample	Description of Sample	Fleece Growth	No. of Bands	Colour of Bands		Width of Bands	Fibre Type						
							Banded	Coloured	White	Intermittent	Bipartite		Total
						cms.					a	b	
A	South Australian Merino. Whole fleece, (i) shoulder, (ii) belly.	12 months 12 cms.	3	Distal	Brown	3·7	i, 36	—	54	6	2	2	100
				Median	White	5·5	ii, 57	32	9	2	—	—	100
				Proximal	Dark grey	3·4							
B	Western Australian Merino. Portion of skin.	9 months 10 cms.	9 3 major, with imposition of narrow bands in median bar	Distal	Light brown, with very pale tips	5·5	72 (a:40) (b:32)	6	8	14	—	—	100
				Median	White	2·9							
				Proximal	Dark brown	2·4							
					(The narrow bands which traverse the median white are brown)	0·2							
C	South Australian Merino. Portion of skin.	9 months 8 cms.	3	Distal	Reddish brown	4·54	86	9	3	2	—	—	100
				Median	White	1·05							
				Proximal	Reddish brown	2·23							
D	Cape Merino. Samples i and ii.	12 months 8 cms.	6 with indication of 7th proximally	Distal	Dark brown	2·4	i, 94 ii, 90	3 2	3 1	— 3	— 4	— —	100 100
				Median	Narrow white	0·1							
					Dark brown	0·1							
					White	0·3							
					Dark brown	0·2							
				Proximal	White	5·1							
E	Coloured Hausa × Shetland cross. Sample.	10 months 12 cms.	5 with indication of 6th proximally	Distal	Black, with dark brown tips	7·4	67	26	3	4	—	—	100
				Median	Grey	1·8							
					Black	0·8							
					Grey	1·2							
				Proximal	Black	0·8							

TABLE 2

not yet completely understood but banding itself is a condition which has been observed in many animals besides sheep, such as rabbits, guinea-pigs, rats and mice. Here the fibres are seldom sufficiently similar in their banding to give the bands of the coat as a whole a clearly defined outline. Dry (15), working on rats and mice, found that adjacent follicles may at the same moment be producing black pigment in the one case and yellow in the other. This is now known to be due to the degree of oxidation of the melanin.

Since all five sheep from which the samples were taken started life as coloured lambs, there is reason to believe that the sheep producing the banded fleece is genetically coloured.

A type of banding is described by Wassin (96) to which he gives the name agouti. This banding is of a genetic nature and involves a light ring towards the tip of dark fibres. In the actual fleece, however, agouti banding was found only in new-born lambs, and was never observed in the adult. Similarly, Roberts and White (83) describe a condition in the coat of some of their coloured experimental lambs in which the tips of a proportion of the fibres are golden brown in colour. In both these cases, however, the fibres affected are birth-coat fibres, together with short, stiff/

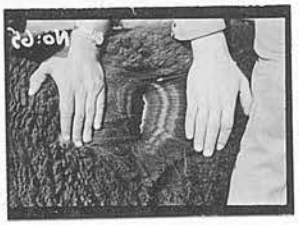


Figure 17. Black ram 'E' with banded wool from flock at Institute of Animal Genetics, Edinburgh.

stiff hairs which are, in all probability, derived from the outer coat. Apart then, from the fact that parti-coloured fibres are involved in all these cases, the banding here described appears to be an entirely different phenomenon.

Fortunately, the ram from which Sample E was taken was available for further study of the banding phenomenon, and has, for the past fifteen months been kept under observation. Quite inconsistently last year's fleece (1930-31) showed no trace of banding, but five of the banded ram's male progeny exhibited the phenomenon, although he was mated to non-banded ewes. There has not been noticed any banding in his female progeny up to date. This seems to offer conclusive evidence that the tendency for banding is a genetic character and as such definitely heritable. It is permissible to postulate that the exhibition of banding depends upon the combination of the necessary genetic factor or factors with some environmental condition. The absence of the latter during 1930-31 in this previously banded ram might account for the absence of the banding in this particular year. A short time ago in taking skin samples from certain sheep another individual, a Welsh cross Badger-faced castrate, was found to be growing a banded wool. A review/

review of prevalent environmental conditions of the last three months was immediately made and it now seems probably that banding may be defined as a "somatic manifestation of a cryptomeric character", using the term "character" in its widest sense. The existence of the character has been rendered possible by an environmental variation. In this particular case the cessation of colour production was apparently due to a protein deficiency in the food, and the renewal of pigmentation coincided with the abundant supply of the necessary protein. This enables one to indicate the importance of the rôle of nutrition in pigmentation. Experiments are to be carried out in which the protein deficiency theory and its effect on pigmentation will be tested. In so far as the genetic significance of banding is concerned, it is not so much the character for banding which is heritable as a tendency by which variation in protein metabolism affects the pigmentary system of one animal more than another.

As far as could be ascertained from this study there is little association between fibre colour and structure, and, as is evidenced by Landauer (51) when such association occurs it is more apparent than real.

IV. DISCUSSION.

In the present state of our knowledge concerning the mechanisms underlying pigment production it is impossible to define fully those laws which govern the activities of the pigmentary system. The conditions under which pigment formation occurs and those which are associated with its inhibition exhibit an extraordinary, wide range of variation, and it is not unusual to find that different animals when exposed to a similar stimulus respond in entirely different ways. The study of this problem of pigmentation probably requires years of further research and at present it is only possible to summarise the results already obtained, and to indicate their significance.

The earliest investigators were mainly concerned with colour in its final exhibition and in systematic descriptive work, colour, occurring as it does in the skin and its appendages, has figured largely as a definitive character. This normal colour which characterizes species is purely genetic and dependent on the fact that a pigmentary system is present, whilst any modification of normal colour has its origin in some stimulation or inhibition of the system itself. The specific colour may therefore undergo various modifications associated with mimicry, seasonal variation/

variation, secondary sex characters, and finally, pathological conditions. In every case the environmental stimulus is dissimilar and yet the underlying physiological principles which initiate the response are the same.

To the naturalist protective mimicry with all its modifications, is a well known phenomenon and, although protective mimicry in the invertebrates is dependent on form as well as colour, in the vertebrates it is largely if not solely an expression of the pigmentary system. There are many well known illustrations of such colour mimicry.

It is logical to believe that natural selection has had as much to do with the fixation of these adapted colours as modern breeding practice has had to do with the establishment of distinct breeds. It is obvious how great a part colour plays in the standardisation of some breeds of horses, pigs, cattle etc.

The colour changes which may accompany seasonal variation are perhaps the most striking of all colour phenomena and are specially well marked in the regions of the Arctic. The arctic fox, the ptarmigan, and Willow grouse, the mountain hare and the stoat are typical examples which illustrate a hiberalbic condition.

The/

The loss of colour is not however always complete and many of the animals retain pigmented acra; e.g. the black tips of the ears of the mountain hare and the black tip to the tail of the stoat.

Amongst the several secondary sex characters, colour is of special importance, particularly in birds where colour dimorphism is often extremely well marked. In addition to this, phases of increased sexual activity are in many cases characterised by definite changes in the colour of the plumage, and during the pregnancy of certain mammals, increased pigmentation of areolae, external genitalia, axillae, and circumorbital skin may occur.

Finally, there is the pigmentation which sometimes accompanies a pathological or abnormal condition in the body. The functioning of the pigmentary system is in this case subject to extreme variation extending from hyper-activity to complete inhibition. The bronzing of the human skin which occurs in Addison's disease is an excellent example of this so called "pathological pigmentation", and has its origin in the dys-functioning of the adrenal bodies.

Even such a brief consideration of some of the various manifestations of colour, indicates how wide is the range of variation in the activities of the pigmentary/

pigmentary system and the present study has tended to show that pigment often has a protective function.* The factors which may influence the pigmentary system, and thus, the ultimate colour, are varied. Apart from the genetic constitution of any one animal or species under consideration, experimental evidence shows that the following:-

Light, (various types of rays)
Temperature changes
Endocrine secretions
Trauma (mechanical, thermic or chemical)
and Certain sarcomatous growths

are each capable of producing a reaction in the pigmentary system.

Intense light may exercise a very deleterious effect, and pigment by absorbing the harmful rays, is able to protect the tissues immediately below. Particularly is this true where the skin is not protected by any covering and is thus directly exposed to the ultra-violet rays. For example, in the elephant and in man an effective pigmentary system produces an even layer of pigment, causing in man what is known as tanning, but where the pigmentogenic cells are not evenly distributed, particularly in the blonde individual, freckling occurs, and where the pigmentary system/

* Admittedly there are instances when this function is not obvious but there is little justification for the belief that pigment is to be regarded as a luxury or waste product.

system is only partially present, the risk of injury from sunburn is increased. (Percival and Stewart, amongst others, have experimentally obtained increased pigment production in human skin after exposure to ultra-violet rays). In naked skinned animals such as the rhinoceros, elephant, hippopotamus and pig the pigmentary system is functional over the whole body and an even layer of pigment is present, protecting the tissues below. In the albino, or white varieties of these animals, however where the pigmentary system is non-existent or non-functioning, sunscald is a common occurrence. This is specially prevalent in the white pig and indicates the importance of pigmentation of the skin. These generalisations must however be modified, and in animals where the body covering is very thick all the year round, or in those not exposed to intense sunlight, there is little need for pigmentation. The muskox, the fox of the Arctics and the snow leopard of Tibet are, for this reason, white skinned.

(There has been no consideration here of the colour changes which occur in some Amphibia and Reptilia on exposure to bright light or after maintenance in darkness since these colour changes have their origin in the fact that the pigment producing cells or melanophores are contractile, and the colour produced/

produced depends on the state of expansion or contraction of these cells).

The rôle of temperature in pigment production is an important one; a layer of pigment, especially in a poorly covered skin, is a useful aid to the heat regulatory mechanism of the body and Schultz has found in the Himalayan rabbit there is a definite temperature threshold below which pigment is produced. Iljin (39) continuing this work determined that the threshold was not the same over the whole body, but varied according to the site. The rapid disappearance of the pigment and the discontinuation of pigment production immediately after restoration of the normal temperature is significant.

When animals which do possess a pigmentary system are injured, either by mechanical, thermic, or chemical means, there is usually a response on the part of the pigmentary system. In these cases it is not always clear why the pigment is produced, but it seems that its function is protective, for injured tissue is less able to withstand harmful light rays, sudden temperature changes, etc., and a deposition of pigment thus acts as a buffer. Such pigmentary activity is usually transient and ceases when tissue repair is perfected. Such a temporary manifestation is/

is seen in the pigmentation of non-pigmented skin which in some cases (See skin transplantation in coloured-haired white skinned mice, p. 49) follows acute trauma. In such areas, the cellular activity concerned with the repair is at a higher level of intensity than that of the normal surrounding skin. It is permissable to suggest that the dopa-positive cells in such a region take part in this intensification of activity. This activity must fundamentally be referred back to an acceleration of local metabolism depending in turn upon the controlling influence of the trophic nerves of that area. Increased pigmentation as a result of thermic irritation occurs in coloured cattle and horses at the site of branding, but unlike the temporary pigmentation induced by various other forms of injury, this pigmentation persists indefinitely. It is suggested that the momentary nature of the stimulus whilst injuring the epidermis sufficiently to arrest the functioning of follicles leaves the skin less protected than formerly and therefore in need of such a substitute as pigment. This adequately explains the continued production of pigment in such areas. Pigmentation may be induced by the subcutaneous injection of irritants such as acetic acid or by the external application of xylol or croton/

croton oil. In these cases the irritants are mere stimuli, completely non-specific.

The tissue injury which characterises a sarcomatous condition may in some cases be accompanied by pigmentation producing what is known as a melanotic sarcoma. The stimulus to pigmentation is again the increased cellular activity.

The main discussion up till now has been concerned with the cause and occurrence of increased pigmentation but depigmentation must also be considered.

There is a stage, at which the pigmentary system completely ceases to function and even though the original stimulus, which initiated pigment production, is removed the system is rendered permanently incapable of pigment production. Hance and Murray (28), have shown this in mice after X-ray treatment and it is possible that in these cases the minute nerve controls of the pigmentogenic cells were destroyed.

The depigmentation which accompanies age may be ascribed to a progressive slowing down of cellular activity and a consequent inability on the part of the cells to produce pigment.

The depigmentation which sometimes follows severe shock/

shock is almost certainly nervous in its origin. Provided the shock has not been so severe as permanently to impair the nerves, pigmentation occurs again as soon as conditions are normal. But where the trophic nerves have been permanently injured, as in horses where chronic pressure necrosis of the skin has produced collar galls, girth galls, saddle spots, etc., the pigment forming ability of the cells of the immediately injured tissue is lost. The same loss of pigment-forming ability which was noted in those tissues exposed to excessive X-rays is seen again after injections in strong concentrations of such substances as thyroxin. Many authors have, of course, failed to get such an extreme response from thyroxin, but in these cases it is clear that the preparations used were too dilute.

The influence of endocrine secretions on pigment production is undoubtedly great and dysfunction of the endocrine organs is often accompanied by altered pigmentation. A possible reason for this close association lies in the fact that the precursor of melanin, tyrosine, may feasibly be the same as that of a particular endocrine secretion and in any case of hypo- or hyper-functioning the supply of tyrosine is diverted to or from the pigmentary system; or, the pigmentation may be due to the stimulus of greater cellular/

cellular activity which exists under pathological conditions. Greenwood (23) Torrey, and Horning (34) amongst others have described the effect of thyroxin on pigmentation in fowls. In some cases pigment production was accelerated, possibly the result of an acceleration of somatic growth characteristic of thyroxin stimulus, and in other cases it was completely inhibited. It is possible that the inhibition of pigment production was due to an overdose or thyroxin shock. It was found that when thyroxin 1:1000 was injected subcutaneously into the young Argenté rabbit, a local affect resulted. Pigment was produced in quantity round the site of the injection and the baby coat was shed to be replaced by typical adult fibres. That this was specifically due to some stimulus evoked by the thyroxin, possibly increased local metabolism, and not mere irritation, was evidenced by the fact that injections of an alkaline solution of the same pH value as the alkaline solvent of thyroxine produced no effect. Huestis and Jocom (35) working on Peromyscus found that intra-peritoneal injections of thyroxin produced no pigmentary change in the coat. There are several probable explanations of the discrepancy between these results and these referred to earlier. Firstly, the amounts/

amounts of thyroxin used may have been inadequate; secondly, the site of injection (peritoneum) may have been too remote from the cutaneous system to have influenced it, or thirdly, the response of animals of differing ages and species may vary.

The pigmentation which accompanies pregnancy is well known and owes its appearance probably to the stimulus of either anterior pituitary hormones or oestrin. This may be correlated directly with the increased metabolic activity which accompanies pregnancy. (Fraser and Wiesner [21]). The plumage changes in birds during periods of sexual activity may thus be directly attributable to the excitation of the sex hormone and, similarly, the colour dimorphism of sex may be of hormonic origin.

In the foregoing brief discussion on the effect of environment on the activities of the pigmentary system an attempt has been made to indicate the labile nature of a system whose activities may be restricted to one of two groups. The first is what could be called the normal group and comprises all those manifestations of colour which are apparently stable, such as breed colours. The second and less familiar group contains all those exhibitions which are abnormal, or,more positively a response to some definite/

definite stimulus, such as the light rays which produce tanning. It is this versatility of the pigmentary system and its extreme sensitivity to external stimuli that make it somewhat difficult to draw the line between normal and abnormal pigmentation; for, pigmentation which is characteristic of one physiological phase is absent during others, so that every individual, whilst conforming more or less to a certain standard characteristic of the whole species is capable of exhibiting a wide range of variation, depending on the stimulus to which it is exposed. To reconcile all these various manifestations of the pigmentary system it is necessary to postulate that, whilst the possession of a pigmentary system is due to a definite genetic factor the opportunity for expression is under an environmental control which is such that whilst in some cases the potentiality of a system may be fully realised, in others it is never explored. Most animals, however, find their level at the line of normality although the range of variation lies within wide limits.

The normal or stable expression of colour, such as it appears in the characterisation of breeds, shows many variations ranging from self colour, through various piebalds and skewbalds patterning and spotting, to albinism, each with its own modifications. Since/

Since it must be acknowledged that definite gene systems are responsible for such distinct characterisations as pattern and since every gradation between albinism and self colour exists, it is not remarkable that selective breeding has been able to fix and maintain such specific colours or patterns as characterise each of the pure breeds of domesticated animals and birds.

Albinism is in its origin pathological, but is often able to adjust itself to the environment and thus become established. The lack of a pigmentary system is certainly more serious in those animals in which the skin is not protected by hair, etc., and specialised adjustments are necessary. For instance, in albino mice which had been shaved in preparation for operative work the tissues below the denuded area, unable to maintain the requisite temperature and unable to produce protecting pigment, showed a marked increase in layers of large vacuolated connective tissue cells, a direct aid to the heat conservance regulatory mechanism of the body. Whilst it cannot be said that albinos in a favourable environment are at a disadvantage when compared with pigmented animals yet in an unfavourable environment, those individuals equipped with a pigmentary system stand a better chance/

chance of survival. Albinism is, or course, a graded condition and the pigmentary system may be present and expressed in varying degrees or present but unexpressed, although in the true albino it is completely lacking.

Is spotting then to be considered as partial albinism and is it permissable to suggest that a piebald individual is verging towards albinism? Beyond the convenience of such terms in description there can be no grounds for making such assumptions. Darbishire in a simple experiment effectually showed this by crossing albinos and piebalds and producing an F_1 of self-coloured individuals.

Patterning and spotting are however, somewhat different in character. The former is due to genetic pattern factors maintained by the inhibition of cellular oxidases in the colourless areas, the latter, whilst it may in extreme cases be of the same nature as patterning, is often the response to an effective stimulus, for a spotted individual may possess a complete although partially quiescent pigmentary system.

Mudge, in an attempt to discover whether or not a chromogen were present in the white portions of the coat of a patterned individual, applied a chemical oxidising agent which would act on the chromogen were it/

it there. His results were negative. More recent work by Onslow, Przibram, Brecher, and Bloch in which skin extracts of patterned animals were tested, demonstrated not the presence or absence of the chromogen, but the presence of an anti-oxidase or inhibitor. In our work with lamb skins this same result appeared, but the in vitro experimentation carried out with dopa permits us to suggest a further explanation. The postulation of the existence of inhibitors and anti-inhibitors to account for either the appearance or suppression of colour is as cumbersome as that of multiple factors in spotting. It is here that the importance of the chemical environment is most apparent, for it has been shown that a variation in pH is sufficient to accelerate or inhibit melanin formation, and might thus constitute an inhibitor or anti-inhibitor. The recognition of this fact may render unnecessary the acceptance of the inhibitor as a specific substance. In an area of skin deficient in intra-cellular oxidase the dopa carried to it by the circulatory system would pass unchanged to a site where oxidase was present and combination would there occur with consequent deposition of melanin. No chromogen would be retained by the cells in the white hairs and Mudge's attempt to demonstrate it was based upon a wrong assumption. Schmalfuss and Barthmeyer(88) have/

have demonstrated the presence of unaltered chromogen in the elytra of the potato beetle which are always heavily pigmented. In vitro experimentation has shown that when dopa is present in excessive quantity and oxidase is limited the precipitation of melanin is likewise curtailed; this is apparently what occurs in Nature. It is interesting to speculate on the amount of unaltered chromogen which may often be present in coloured skin and hair.

How often lack of pigment is to be attributed to the action of inhibitors and how often to an inadequacy in the pigmentary system is difficult to state. Many workers have attempted by means of the skin extract method to confirm or refute the existence of inhibitors, but it is possible that the method will have to be reinforced by the frozen section technique before the results can be accepted. In the tests which we carried out on sheep it was only possible to use the latter method. The lack of reaction in skin sections of a white spot from a black lamb is puzzling especially when it is remembered how complete were the opportunities which were offered for melanin deposition. It is inferred therefore that the cells of that white tissue were not potentially pigment producing and that a genetic factor rendered melanin formation/

formation impossible. The intense reaction which was obtained in the white skin of the reversed badger faced sheep demonstrates conclusively the capacity for localised quiescence possessed by the pigmentary system, and if the ancestral sheep from which modern breeds have sprung were brown then the white of to-day is the result of an inhibition of such colour and it is significant that the dopa reaction should enable one to demonstrate that even a white sheep may be a potentially coloured one.

That an inhibitor need not necessarily be a specific substance, but may be resolved into a pH variant, a temperature change or a quantitative change in either oxidase or chromogen (i.e. of nutritional origin) finds much supporting evidence. Przibram stresses the fact that the pH of the cells of the retina of the eye is slightly higher than in cells of other parts of the eye, so that once the normal melanin deposition has occurred in the choroid, further pigmentation which might possibly interfere with vision, is impossible. He inclines definitely to the view that acidosis may be responsible for albinism. We admit that even though the dopa solution itself is standardised as carefully as possible it is obviously impossible similarly to control the intra-cellular environment/

environment and that therefore the inhibiting factor may be there. In age, accompanied by greying, acidosis is present and cellular activity is also reduced. Both these factors militate against melanin formation.

The marked difference in the results obtained from adult and lamb skins sections when stained with dopa are suggestive. The reaction in the adult sections, although maintained, is considerably lessened and there is no surplus of either chromogen or oxidase. In coloured lambs, or apparently nearly all young animals, the supply of both, is prodigal and offers a simple explanation for the fact that so many newborn animals are much darker than the adults. (cf. Suffolk). The adjustments which are necessary when the young animal assumes independent existence are necessarily of a far-reaching nature. The abundant source of supply of protein, available for a plentiful chromogen production is, as age increases, diverted to other purposes, and nuclear activity becomes concerned with much more than the manufacture of oxidases. Naturally these adjustments take time and so it is that in the Suffolk, for instance, the general adult fleece characters are assumed only about the end of the third month. The Argenté rabbits which are born black fail to show any silvering till ten/

till ten days or so after birth and the loss of the pigmented coat is progressive. A speeding up of metabolism by means of injections such as thyroxin results in an earlier assumption of the adult coloration but this result is due not to a direct effect of the thyroxin on the pigmentary system but to increased general cellular and metabolic activity. The coloured tips of the otherwise white wool fibres of the Suffolk find a similar explanation, but it is more difficult to determine those factors which govern the seasonal production of black kemps. It seems that the conditions which first permitted pigment formation in the fibres of the coat persist for the kemp follicles or that they have a lower threshold of response. Schultz attempted to show that the varying levels at which fibres were produced influenced the pigmentation but in the sheep, at least, this explanation cannot be entirely accepted, for similar kemp and wool fibres are found at irregular depths in the skin.

The enormous variation in fibre pigmentation is in itself confirmatory evidence of the erratic activity of the germinal pigment cells. Even in heavily pigmented fibres the quantitative deposition of pigment varies along the length of the fibre, and in poorly pigmented fibres this is even more marked. In white-fleeced/

fleeced sheep which are basically coloured the occurrence of colour tipped fibres ought not to occasion surprise, since the colour is only an obvious proof of some activity on the part of the pigmentary system. A parallel example is afforded by the microscopic detection of pigment granules in many fibres which to the naked eye appear white.

If we accept the function of pigment to be one of protection then the pigmentation of the so called Schultz reaction may well be explained. It is a direct response on the part of the pigmentary system to maintain, by means of the deposition of pigment, the efficiency of the heat regulatory mechanism of the body. Kopéc (46) has shown that in the Himalayan rabbit the low temperature precedes a degree of alkalinity which offers a suitable stimulus for pigment production. Some animals are susceptible to temperature influences and others are not and in connection with this it may be noted that the Schultz reaction has never been recorded for sheep.

It is accepted that the rôle of endocrine secretion in pigmentation is an important one, and it has been shown that pituitrin, thyroxin, adrenalin and sex hormones may at times influence pigment production. It must be remembered at the outset however that only those/

those cells which are potentially pigment producing can produce pigment and no endocrine secretion is effective when a pigmentary system is lacking. Hutt has shown that whilst a small quantity of thyroxin may increase pigmentation in the fowl, larger sub-toxic doses completely inhibit it. In the latter case the dose may have paralysed the trophic nerves of the pigment cells, or the injection may have disturbed the intracellular pH of the dopa positive cells which were then unable to function normally. The production of pigment after recovery from the shock dosage would seem to support this.

Torrey and Horning suggest that the depigmentation which they observed in the fowls to which thyroid had been fed, was due to disturbed nutrition and that the resultant nutritive deficiency in the cells was responsible for the lack of pigment production. This would seem to be in accordance with our recent observations on the growth of the banded fleece. Although there was no experimental interference in this latter case, natural environmental conditions were such that there was a phase of protein deficiency when colourless wool was produced. It must be admitted at the same time that other coloured sheep exposed to a similar environment showed no such inhibition of pigmentary activity.

A/

A comparison with results obtained in the study of pigment behaviour in Amphibia Pisces and Reptilia [Hogben (33) et alia] is somewhat arbitrary since in no case in Mammalia can the response be so rapid. In the lower vertebrates, colour manifestations are greatly influenced and indeed practically controlled by the fact that the melanophores are contractile and the colour therefore mainly depends on the state of contraction or expansion of these cells.

If on the authority of the biochemist we are to accept a common origin for melanin precursor and adrenalin, then the pathological bronzing of the human skin which occurs in Addison's disease is easily explicable. The experimental adrenalectomy carried out on mice, which, it was hoped would throw further light on this point, was unfortunately negative in result. It was found that six months after operation compensatory growth of adrenal tissue had taken place in the region of the sympathetic ganglia and that therefore no surplus melanin or adrenalin precursor was available to intensify pigmentation.

In considering the influence of sex hormones on the activities of the pigmentary system, it is significant that the most intense degrees of pigmentation are specially apparent in the regions of greatest/

greatest growth. Lipschutz (52), using the fact that in guinea pig pregnancy, pigmentation of the areolae and nipples occurred, investigated the influence of ovarian grafts on corresponding areas in the male. He found that pigment production which did not normally occur in those areas could be stimulated by implantation of ovarian tissue; this was limited, however, by the colour nature of the surrounding tissues. For instance, nipples in a colourless area in piebald animals never, whether in male or female showed pigmentation. This supports Bloch's theory, that pigment formation is a local reaction in a predisposed site. Finally, the growth processes set up in the mammary gland either produce or condition the factors responsible for pigmentation, and pigmentation occurs only if the potentiality for pigment formation is inherent in the parts concerned. It is feasible to suppose that under such conditions another stimulus other than that provided by endocrine secretion could produce a similar pigmentation, and that therefore the influence of endocrine action is limited to its power as a non-specific stimulus to cellular activity. The relation between proliferation and pigmentation is further suggested in the case of a unilaterally castrated rabbit in which the scrotum on the same side as the remaining hypertrophied testicle showed marked pigmentation/

pigmentation. It is admitted, however, that *per se* this is not conclusive evidence.

In reviewing the whole problem from the practical point of view it seems inevitable that the unit in pigment production must be taken to mean the individual melanogenic cell. In the sheep, this is of especial importance, since it indicates that it is not possible to regard the fleece as the unit in pigmentation, nor the fibres composing the fleece (whether taken singly or collectively in areas) but it is the activity of each melanogenic cell in the follicle which determines the production or non-production of pigment. What is it then that determines whether a follicle shall or shall not produce a pigmented fibre?

For pigment production there must be:-

(a) That genetic characterisation which permits pigment production.
(b) Dopa positive cells which in the presence of dopa and an oxidase or oxidases can precipitate melanin.
(c) Dopa circulating in the blood stream.
(d) Oxidase or oxidases elaborated in the dopa-positive cells.
(e) Dopa positive cells in the bulb of each particular follicle, regardless of whether or not they occur elsewhere, *e.g.* in the epidermis, root sheath, etc.
(f) A government of the chemical processes outlined above which, in the light of our present knowledge, we can only postulate is supplied by the ramifications of the trophic nerves or possibly by the Golgi apparatus which those dopa positive cells possess.

Are/

Are there, then, any ways in which the sheep-breeder may hope in the near future to control the colour of the fleece of his sheep without in any way disturbing his other markets? Several possibilities may be discussed.

In the review of sheep breeds given it was shown that in every white breed pigment is demonstrable in some part of the fleece. By care in selection, the breeder has restricted the amount of this colour to a minimum. Just how successful he has been is indicated by the whiteness or otherwise of the wool produced by any particular breed, when considered in bulk. The estimation of this success is at present difficult since the repeated sorting which wool undergoes prevents one from forming a real conception as to its original colour character. The total amount of coloured wool removed during the various sorting processes must be considerable, but unfortunately the percentage is not available.* All breeders agree that much coloured wool remains in clips. Entire elimination of coloured wool may be possible if the present methods of breeding are continued and intensified, but, if the breeder's efforts and the progress he has made are now static, he will require a reinforcement of his breeding methods based upon a deeper/

* In the Merino, coloured fibres are only rarely observed in the living animal and it is not till the wool has undergone a certain amount of processing that they become obvious.

deeper and more comprehensive knowledge of the scientific laws underlying the genetics of colour.

Dry (11) in his work on "The Genetics of the Wensleydale Breed of Sheep" has already shown how the production of the undesirable black lambs may be controlled, provided the breeder is willing to sacrifice his emphatic insistence on the blue face and ear colour.

(The use of a simple chemical test in genetic analyses may be evolved by an amplification of the Dopa work, and it is not impossible that observation of the reaction in sections from the lamb will afford an indication of the transmissable colour potentialities of that particular individual).

It is not claimed here, however, that genetics alone will ever, other than by a profound disturbance of the settled practice of breeding, entirely eliminate colour from all the breeds of sheep, many of which are destined to furnish excellent mutton rather than wool. Inevitably other methods of controlling colour must be sought. Either the manufacturer will be able by a more extended use of chemical processing, to bleach all fibres, coloured and otherwise, to a uniform colour, or some means will be found of controlling by physiological methods, the production of pigment in the/

the living sheep. Grave difficulties present themselves but it is impossible to believe that they are insuperable.

The application in routine breeding practice of measures with a physiological basis which would neutralise the action of the circulating dopa in the blood, of oxidase production by the skin cells, or block the interaction of the constituents of the pigmentary system and thus prevent the precipitation of melanin, would solve the problem. Further research may be destined to accomplish this.

V. SUMMARY.

1. A description of the mammalian pigmentary system is given together with an outline of the mechanism of melanin production and those factors which influence or control it.

2. The production of melanin by chemico-physical interaction between circulating 3:4 dihydroxyphenylalanine (dopa) in the blood stream and an enzyme or enzymes of intra-cellular origin is accepted, but it is emphasised that in the interaction there are numerous opportunities for physiological interference which inhibit melanin deposition.

3. The morphology and disposition of pigment in wool diffuse and granular, is described, and it is postulated that only one pigment exists and that all animal colours are the result of a modification of this one pigment.

4. It is shown that melanin once formed is very stable and that when it occurs in fibres, wool or hair, which are keratinized, it is no longer capable of being influenced by physiological conditions.

5. The current theories of the origin of melanin are discussed and evidence supporting the nuclear origin of oxidising enzymes is given. Nuclear caps are recorded and described in the epidermal cells and wool fibres of the sheep.

6. It is demonstrated that in the sheep some epidermal cells are more potentially pigment-producing than others, and that a gradation exists between the cell which cannot produce pigment and that which produces it to a maximum degree.

7. A series of "in vitro" experiments planned to standardise the dopa reaction is described, and the results of staining sections of lamb and adult sheep skin with dopa are given. It is suggested that the Dopa reaction may be used as a simple chemical test in genetic analysis.

8. A study was made of the individuality of coloured and colourless mouse skin after transplantation to a different environment and it was found that pigment cells once differentiated retain their genetic characterisation. Where, however, coloured skin was grafted on to an albino mouse the/

mouse the production of melanin ceased owing to the lack of the necessary chromogen. On the other hand coloured skin grafted on to a white area in a piebald individual retained its pigmentation.

9. An elaborated technique for skin transplantation, which was devised in the course of these experiments, is described in detail.

10. The Schultz reaction is recorded for piebald and coloured mice used in these experiments.

11. The more important breeds of sheep are briefly reviewed with special reference to their colour characteristics, and the desirability for an understanding of the pigmentary system in sheep is emphasised.

12. A short account is given of some economic aspects of colour in the fleece. The necessity for the elimination of scattered fibres in white wool and the eradication of black lambs from pedigreed flocks is stressed. In connection with this problem special mention is made of the Merino.

13. The results of a microscopic study made of the colour transition shown by the fleece of the Suffolk from birth to maturity are given. It is shown that differences in colour tones in this wool are partly quantitative and partly due to varying physical structure. It is demonstrated that the physical structure of the fibre cuticle obviates the possibility of pigment inclusions.

14. It was not possible definitely to associate colour and structure.

15. An explanation for the sporadic occurrence of coloured fibres in an otherwise white fleece is attempted, and it is pointed out that however else the pigmentary system of the Suffolk may be stimulated, thresholds of irritation conditioned by temperature need not be considered as possible stimuli.

16. The phenomenon of colour banding in the fleece of the sheep is described and a possible explanation for its appearance is suggested.

17. Finally the various expressions of the pigmentary system are discussed and hypotheses are advanced to account for variation. The results of the study suggest that pigment has a definite protective function in the body.

18. From the evidence collected it is logical to postulate that the activity of the pigmentary system is intimately association with, or dependent on cutaneous metabolic activity and that these factors which modify the activity of the pigmentary system do so by influencing skin metabolism, and are non-specific.

19. Only cells with a genetic potentiality for pigment production are able to be influenced by the activities of the pigmentary system.

20. It is conclusively shown that the dopa-positive cell is the unit of pigment production in the body.

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